Usnic acid, a secondary metabolite of lichens and its effect on in vitro digestibility in reindeer

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Summary: Usnic acid, a common secondary metabolite in preferred lichens by reindeer and caribou, has been tested for its effect on In Vitro Dry Matter Digestibility (IVDMD) using inocula from four reindeer. When Cladonia alpestris (stellaris (Opic)) was used as substrate and reindeer rumen liquor as media of incubation together with usnic acid, digestibility was considerably enhanced. This was also true for a lower preferred lichen Stereocaulon paschale (L.), but the effect was less pronounced. The results suggest that reindeer host some rumen microorganism able to metabolize lichen secondary metabolites.

Key words: Usnic acid, Cladonia stellaris, Stereocaulon paschale, digestibility, reindeer

Introduction
Lichens produce a diverse array of secondary metabolites which differ in chemical structure and biological activity. These compounds have been used in taxonomic classification of lichen species and as sources of commercial products (Vartiainen 1949, 1973, Culbertson et al. 1983). The ecological and nutritional role of these compounds is not well understood for lichens and interacting organisms. The idea of some ecological role of lichen secondary metabolites dates back to the 19th century when these substances were first isolated. Backman (1890) and Zukal (1895) suggested that these substances might be important as chemical defenses to protect the plants against herbivores. Few recent studies have focused on this aspect and the reports are largely anecdotal (Rundel 1978, Lawrey 1980, Truell et al. 1980). However, although several mammalian herbivores such as voles (Microtinae) and squirrels (Squiridae) include lichens as a minor component in their diet, only reindeer and caribou use it extensively (Andreev 1977; Gaare et al. 1977; Klein 1980, 1982). Lichens are usually poorly digested by unadapted herbivores, but show relatively high digestibility in reindeer and caribou (Prestegge 1954; Nordfelt et al. 1961; Jacobsen and Skjenneberg 1975; Klein 1982; Jenks and Leslie 1988). This suggests that reindeer and caribou have specialized microorganisms in the rumen capable of handling lichen substances which are absent in other mammalian herbivores.

The lichen genera that normally comprise the major portion of the winter diet of reindeer and caribou are Cladonia spp. and Cetraria spp. These species are high in digestible carbohydrates facilitating high rate of volatile free fatty acid production by rumen microorganisms, but are low in nitrogen (Thomas et al. 1984). Nitrogen-
fixing lichens such as Peltigera spp. and Stereocaulon spp. are occasionally consumed, although the animals' preference for these in feeding trials is low (Holleman and Luick 1977; Palo 1981; Klein 1982). These species show comparatively low digestibilities in vitro, in spite of higher concentrations of nitrogen (White et al. 1980). On the other hand, specific lichen secondary metabolites might have a significant effect on microbial growth and substrate utilization due to antimicrobial effects (Palo and Lundberg 1992). It could be assumed that reindeer and caribou circumvent this effect by inhabiting unique microorganisms in the rumen which are able to metabolize these compounds. A first test of this assumption is to study the effect by isolated compounds on in vitro digestibility. The effect on digestion by metabolites characterized for preferred lichens such as usnic acid, rangiformic acid, etc. may have none or a direct stimulatory effect on digestibility and feed intake, while lower ranked lichen species such as Peltigera spp. species may be metabolized at a slower rate due to antibacterial effects by their specific compounds. Here I report results from a study investigating the effect of usnic acid, a lichen secondary metabolite from preferred lichens, on reindeer in vitro digestibility.

Material and methods

Four individually caged semi-domesticated male reindeer (Rangifer tarandus L.) were used in the experiment which was performed in February 1991 at the field station Kuolpavaare, Norrbotten, Sweden. The reindeer was 21 months old with body masses ranging from 52 kg to 59 kg during the trial. The animals were fed a ration of lichen mixed with pellets in the amounts 0.9 kg/animal*day and 1.4 kg/animal*day respectively (Renfor, Fori, Holmsund, Sweden), during 20 days and on only lichens five days before the experiment. Two of the animals were fed Cladonia alpestris as a single ration and two were fed a mixture of C. alpestris, C. rangiferina and Stereocaulon paschale (3:1:1) ad libitum.

On the fifth day the reindeer were killed and rumen content, approximately 200 ml from each animal, was collected into preheated thermos flasks which were then sealed. The samples were filtered through a cloth within 30 minutes from the slaughter and 20 ml of filtered rumen liquor from each animal were diluted to 1000 ml with buffer solution, containing NaHCO₃ (8.5g), K₂HPO₄(5.8g), (NH₄)₂HPO₄(0.5g), NaCl(1.0g), MgSO₄·7H₂O(0.5g), FeSO₄·7H₂O (0.01g) and CaCl(0.1g) which were preheated to 38°C and saturated with CO₂. The in vitro procedure is a modified Tilley and Terry (1963) incubation, excluding the pepsin-HCL treatment (Den Braaver and Eriksson 1967).

For In Vitro Dry Matter Digestibility (IVDMD) measurements, glass filter tubes with a pore size of the filter of 200 um were used (Lindgren 1979). Each tube contained 500 mg dry mass of either C. alpestris or S. paschale.

Usnic acid (SIGMA, U7876) was dissolved in 96% ethanol giving a stock solution. From this solution usnic acid was added to tubes containing either of the different substrates, giving a concentration of 10 mg/g of plant dry mass in excess of that naturally in C. alpestris. The normal concentration of usnic acid found in this species is in the range 1-2% of dry matter (Lakso and Gustafsson 1952). Controls were treated with pure ethanol and all treatments were done in triplicates. The tubes were then dried for 12 h at 40°C to remove the solvent (Palo 1985). The glass filter tubes were filled with 50 ml of the diluted rumen solution from each donor animal, sealed with rubber stoppers and incubated at 38°C for 96h which corresponds with maximum digestibility (Lindgren 1979). The tubes were shaken rigorously for about 30 seconds every fourth hour during the first 12 hours of incubation. After incubation the tubes were filtered, washed with distilled water and acetone, dried at 105°C for 12 h and weighed.

Statistical analysis was done by analysis of variance (ANOVA) with digestibility as dependent variable, animal and treatment as categorical variables. A Tukey HSD post-hoc test was performed to identify significant effects by treatment (SYSTAT Inc.). Probability level for significance was set at p < 0.05.

Results

No large differences in feed intake and body mass between diet groups were apparent during the period. The IVDMD of untreated samples (controls) nor samples treated with usnic acid differed between animals, justifying pooled data from animals of different feeding regimes, giving a sample size of 4 replicates (ANOVA F=0.785, P=0.507, N=4).

The mean IVDMD in the reindeer of pooled material is 42 % (SD. 8.2) for C. alpestris and 32
Figure 1. In vitro matter digestibility of C. alpestris and S. paschale treated with usnic acid compared to untreated plants using rumen liquor from reindeer. Mean and SD, N=12, *** = P < 0.001, n.s = not significant.

% (SD, 4.4) for S. paschale (Figure 1). However, the difference between the two lichen species was not statistically significant (ANOVA, Tukey HSD test, P=0.989, N=4).

Treatment of the material with usnic acid stimulated IVDMD for both lichen species, but the effect was most pronounced for C. alpestris (ANOVA, Tukey HSD test, P=0.001, N=4), while addition of usnic acid to S. paschale had no significant effect (ANOVA, Tukey HSD test, P=0.145, N=4). IVDMD of C. alpestris after treatment with usnic acid increased to 72 % (SD=20.1) and S. paschale increased to 44 % (SD=15.1). This difference between the two species is statistically significant (ANOVA, Tukey HSD, P=0.001, N=4).

Discussion
Usnic acid is one of the most common secondary metabolites found in lichens (Culberson 1977). It occurs in both Cladonia alpestris and Cetraria nivalis, but not in Stereocaulon spp. (Laakso and Gustafsson 1952, Rundel 1978). Several other compounds such as perlatalic acid, rangiformic acid and ventosic acid have more recently been identified in these species (Santesson 1967, Åkermark 1967). Stereocaulon species lack usnic acid but contain substances such as atranorin (Rundel 1978). The ecological and nutritional role of these compounds are largely unknown, but the results presented here suggest an effect on digestion in ruminants. Such an effect on digestion was indicated by White and Trudell (1980) when comparing in vitro and nylon bag digestibilities of preferred and non-preferred lichens. Here the reindeer apparently is better adapted to some of these compounds than other animals. The stimulation of digestibility in reindeer is surprising since secondary compounds in lichen show allelopathic effects.

Acknowledgement
I thank P. M. Utsi and N. Nutti for assistance in the field, collection of lichens and care taking of animals. N. Tyler gave valuable comments on the manuscript. The Department of Animal Nutrition and Manage-
References


Manuscript accepted 25 August, 1992.