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 prefered 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 liqour as media of incubation together with usnic acid, digestibility was considerably enhanced. This was also true for a lower prefered lichen *Stereocaulon paschale* (L.), but the effect was less pronounced. The results suggest that reindeer host some rumen microorganism able to metabolize lichen secondary metabolities.

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

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Introduction

Lichens produce of 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 (Vartia 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 (Presthegge 1954; Nordfelt *et al.* 1961; Jacobsen and Skjenneberg 1975; Klein 1982; Jenks and Leslie 1988). This suggest that reindeer and caribou have specialized microorganisms in the rumen capable of handling lichen substances which are absent in other mammalien 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 faciliating 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 inhabit 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 prefered 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 prefered 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 liquour from each animal were diluted to 1000 ml with buffer solution, containing $NaHCO_3$ (8.5g), K_2HPO_4(5.8g), $(NH_4)_2HPO_4$(0.5g), NaCl(1.0g), $MgSO_4*7H_2O$(0.5g), $FeSO_4*7H_2O$ (0.01g) and CaCl(0.1g) which were preheated to 38°C and saturated with CO_2. 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 disolved 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 (Laakso 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 categorial 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

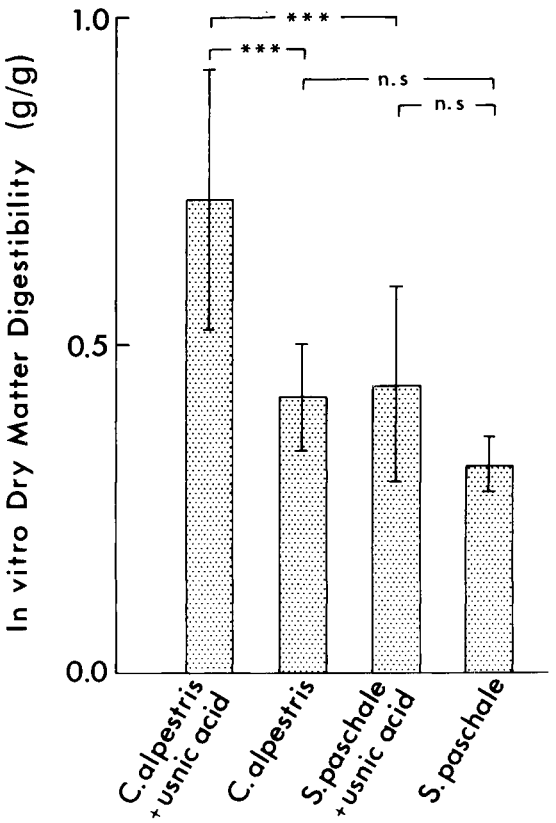

Figure 1. *In vitro* matter digestibility of *C. alpestris* and *S. paschale* treated with usnic acid compared to untreated plants using rumen liquor from reindeer. Mean and SD, N=12, ***=P < 0.001, n.s=not significant.

% (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 coumpounds such as perlatolic 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 suprising since secondary compounds in lichen show allelopathic effects. For instance, usnic acid in *Cladonia* spp. inhibit soil bacterial decomposition rates, growth of fungi and *Pinus sylvestris* seedlings (Vartia 1949, 1973; Asashina and Shibata 1971, Brown and Mikkola 1974). It is reported that lichens are not digested to a large extent by rumen liquor from reindeer not eating lichens and that other mammalian herbivores have a poor ability (Presthegge 1954; Person *et al.* 1975; White and Trudell 1980; Trudell *et al.* 1981, Jenks and Leslie 1988). This suggest that reindeer and caribou feeding on lichens host some microbial species especially capable to handle secondary metabolites of preferred lichen species. For example, addition of usnic acid to hay and incubated with inocula from sheep resulted in a depression of IVDMD. In that experiment the IVDMD dropped from 75 % (SD=0.6) in control to 60 % (SD=3.6) in those containing usnic acid (Palo unpublished data). These findings and the present results support the idea that secondary metabolites of preferred lichens stimulate digestibility in the reindeer. My opinion is that studies of lichen secondary chemistry in relation to nutrition opens new avenues to the interesting field of interaction between reindeer and its food resources.

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