Roe deer *(Capreolus capreolus,* L.): properties of parotid and mixed saliva

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Abstract: The total flow volume, flow rate contents of proteins and of several ions (Na+, K+, Ca2+, inorganic phosphate) and tannin-binding properties of both parotid and mixed saliva were measured in sheep, fallow deer and roe deer.

Introduction: Ruminants represent a vast group of species that consists of three different feeding types: (i) Concentrate selectors (CS), (ii) Intermediates (IM) and (iii) Grazers (GR) (Hofmann and Stewart 1972). One characteristic of CS (e.g. roe deer) diet is a high tannin content (Tixier et al. 1997).

Methods and Materials: *Collection of saliva:* A) parotid: special catheters were introduced into both parotid ducts from the buccal vestibule in anaesthetized animals (Göritz et al. 1994), followed by intra-glandular administration of pilocarpine-hydrochloride. B) mixed saliva was collected by outflow from the mouth. *Electrolytes:* Ca2+ and Pi were measured using test kits (Boehringer, Germany). The Electrolyte Analyzer 984-8 (AVL, Bad Homburg, Germany) was used for analysis of Na+ and K+ concentrations (Breves et al.,1987). *Isolation of salivary proteins:* saliva was mixed with 10% TCA, incubated and centrifuged. The supernatant was collected, titrated to pH 8.2, ultra-filtered and its concentration determined. *Tannin-binding:* Per well a 100µ1 preincubation-mix was prepared (90mM Tris-HCL, 18µM CaCI2, pH 8.2, 100µg/ml trypsin solution, 100µg/ml tannin solution, 10-40µg/ml protein solution) .After 15min preincubation at RT and addition of 100µ,l 1mM N(-benzoyl-DL-arginine-p-nitroanilide, the mixture was incubated for 20 min at 37°C. Trypsin was inhibited by adding 50µ1 of 3% acetic acid. Two controls were utilized, one without tannin and binding protein, the other without binding protein. *Gelfiltration:* We used an FPLC(-system equipped with a 24ml Superdex(75 column (Pharmacia). Running buffer: 1x PBS pH 8.2. Monitoring: at 280nm.

Results: In roe deer the relative parotid saliva volume in ml/kg body mass/10 min was 3.9 times higher than in fallow deer and 4.6 times higher than in sheep, respectively. Roe deer had the highest relative secretion of mixed saliva too. Comparison showed a similar ratio between roe deer and fallow deer (3.9: 1). Na+ and Pı concentrations were higher in parotid saliva of all three species, whereas K+ and Ca2+ concentrations were higher in mixed saliva. Fallow deer (IM) had the highest Pı-concentrations in both saliva types. Roe deer parotids secreted the most protein with respect to body mass, about 4 times more than the other ruminants included in this study. In mixed saliva, major proteins were found at 39.8 kDa and 24.5 kDa. In parotid saliova, major peaks were at 60 kDa, 41.5 kDa and 32 kDa. *Tannin-binding studies:* Parotid proteins bound about 1.8 times more tannic acid and about 1.5 times more quebracho than proteins from mixed saliva. Mixed saliva bound 6.9 times more tannic acid and 1.5 times more quebracho than BSA. TCA-soluble parotid proteins (>6 kDa) had the best binding to tannins. The lowest binding was found in the untreated protein fraction with proteins <6 kDa. A given amount of TCA-soluble protein (5µg/ml) was treated with increasing concentrations of tannic acid. A comparison

between TCA-soluble proteins (MW > 6 kDa) from parotid and mixed saliva shows, that the capacity of parotid salivary proteins to bind tannins is much larger than of proteins derived from mixed saliva.

Conclusion: Apparently, roe deer paritid saliva contains proteins, which are capable of binding large amounts of tannins. The lack of tannin-binding in the fraction of parotid proteins smaller than 6 kDa leads to the assumption, that apparently roe deer do not have histatins, at least not predominantly involved in tannin detoxification. Whether or not the proteins in the roe deer parotid saliva are proline- or histidine-rich proteins or completely different in structure and whether or not there is only one, or perhaps a group of tannin-binding proteins, that bind different sets of tannins, needs to be further elucidated.

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