

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

Joerns Fickel and Britta A. Joest, Department of Evolutionary Genetics, Institute for Zoo Biology and Wildlife Research, Alfred-Kowalke-StraBe 17, D-10315 Berlin, Germany

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 (Görizt 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 100µl preincubation-mix was prepared (90mM Tris-HCL, 18µM CaCl₂, pH 8.2, 100µg/ml trypsin solution, 100µg/ml tannin solution, 10-40µg/ml protein solution) .After 15min preincubation at RT and addition of 100µl 1mM N(-benzoyl-DL-arginine-p-nitroanilide, the mixture was incubated for 20 min at 37°C. Trypsin was inhibited by adding 50µl 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 Pi 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 P_i-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 saliva, 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 parotid 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.

References

Breves G. , Rosenhagen C., Hoeller, H. Saliva secretion of inorganic phosphorus in phosphorus-depleted sheep. **JOURNAL OF VETERINARY MEDICINE A** 34: 42-47, 1987

Göritz, F., Hildebrandt, T., Hofmann, R.R., Pitra, C. Comparative salivary studies using a new technique yielding uncontaminated saliva from CS, IM and GR ruminant species.

PROCEEDINGS OF THE SOCIETY FOR NUTRITION AND PHYSIOLOGY 3: 319, 1994

Hofmann, R.R., Stewart, D.R.M. Grazer or browser: a classification based the stomach structure and feeding habits of East African **MAMMALIA** 36: 226-240 1972

Tiexier, H., Duncan, P., Scehovic, J., Yani, A., Gleizes, M., Lila, M. Food selection by European Roe deer (*Capreolus capreolus*): effects of chemistry, and consequences for the nutritional value of their diets. **JOURNAL OF ZOOLOGY, LONDON** 242: 229-245, 1997

