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Factors affecting the identification of stone marten (*Martes foina*) and pine marten (*Martes martes*) faeces by bile acid content

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Keywords: diet, faecal bile acids, faeces, *Martes foina*, *Martes martes*, thin-layer chromatography
Schlüsselwörter: Nahrung, Kot-Gallensäuren, *Martes foina*, *Martes martes*, Dünnschichtchromatographie

Summary

Distinguishing faeces of the stone marten from those of the pine marten is often a problem when both species occur sympatricly. We tested whether faeces from both species can be identified by thin-layer chromatography of faecal bile acids. Further the effects of diet composition and of food restriction on faecal bile acid concentration were evaluated by feeding experiments with two captured stone and pine martens and by assessing the diet of two wild stone martens. It was not possible to distinguish between faeces from stone and pine marten by their bile acid contents. All tested bile acids were detected in the faeces of both species. We also found no sufficient inter-specific differences of faecal bile acid concentration. The bile acid concentration in the faeces of both species was strongly influenced by diet composition and by the quantity of ingested food.

Zusammenfassung

Die Unterscheidung von Steinmarder- und Baummarderkotproben ist oft problematisch, wenn beide Arten sympatrisch vorkommen. Wir haben untersucht, ob sich Kotproben der beiden Arten anhand von Dünnschichtchromatographie der enthaltenen Gallensäuren identifizieren lassen. Weiterhin wurden die Einflüsse von Nahrungszusammensetzung und -verknappung auf die im Kot enthaltene Gallsäurenkonzentration mittels Fütterungsexperimenten an zwei in Gefangenschaft gehaltenen Stein- und Baummardern, sowie durch Nahrungsuntersuchungen an zwei freilebenden Steinmardern bewertet. Es war nicht möglich, Kotproben von Stein- und Baummarder anhand ihres Gallsäuregehaltes zu unterscheiden. Alle getesteten Gallensäuren waren im Kot beider Arten nachweisbar. Außerdem fanden wir keine hinreichenden interspezifischen Unterschiede bezüglich der Konzentration der im Kot enthaltenen Gallsäuren. Die Gallsäurekonzentrationen im Kot beider Arten war vielmehr stark von der Nahrungszusammensetzung und der Menge aufgenommener Nahrung abhängig.

1. Introduction

Faeces of sympatric carnivores like the stone marten (*Martes foina*) and the pine marten (*Martes martes*) are difficult to distinguish between, because they are similar in size, shape, and content of food remains. One possible approach is to analyse faecal bile acids, which have shown to be specific in occurrence and concentration in some carnivore species (MAJOR et al. 1980; JOHNSON et al. 1981, 1984; RAY & HUTTERER 1995). In contrast, studies on other carnivores found that dietary factors affect faecal bile acid patterns and prevent a specific determination (QUINN & JACKMAN 1994, JIMÉNEZ et al. 1996). In the present study we identified faecal bile acids of stone marten and pine marten using thin-layer chromatography. Our objectives were to determine (1) whether faeces of the stone marten and pine marten are distinguishable by analysing the bile acid contents and (2) whether food restriction and diet composition affect the concentration of faecal bile acids.

2. Material and Methods

2.1 Animals and sample collection

Faeces from two wild and two captive stone martens, and from two captive pine martens were analysed. The captive animals were given a variable diet consisting of meat, cereal, fruit and additional minerals. All faeces were removed daily from the enclosures. Thirty-four faecal samples each from the two captive stone martens and from the two captive pine martens were analysed. Faecal samples from the two wild stone martens (n = 22+8) were collected from their day resting sites during a period of 10 months, when both individuals were radio-tracked (see RÖDEL et al. 1998). For each pellet the percentage volume of included vegetarian food remains (fruits and other plant material) was estimated.

2.2 Thin-layer chromatography

Thin-layer chromatography was used following the procedures described by MAJOR et al. (1980). Pre-coated TLC plates (Merck, Germany) were used, as recommended by JIMÉNEZ et al. (1996). On each plate, the samples were run together with a standard mixture (concentration: 5 mg/ml) of purified cholic (CA), cholic methyl ester (CAME), deoxycholic (DOCA), dehydrocholic (DHCA) and lithocholic (LCA) acid (Sigma Bile Acid Kit, USA). Visualisation of steroid bands was accomplished by spraying with 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (JOHNSON et al. 1986). The relative concentrations of the various bile acids were determined visually under long-wave ultraviolet light by an investigator naive to the experiments. We defined four concentration levels (0 = absent, 1 = low, 2 = medium, 3 = high) based on the size and intensity of the detected bands. For each bile acid, the intensity of the corresponding standard band represented a 'high' concentration.

2.3 Data analysis

The average bile acid concentration of the five detected steroids CAME, CDOCA, DOCA, DHCA, and LCA was determined as the frequency (in %) of occurring concentration levels for the total number of faecal samples of each species. Inter-specific similarity of concentration level patterns was determined for each bile acid using Schoener's index (SCHOENER 1968) calculated as:

$$D = 1 - \tfrac{1}{2} \sum | p_{ji} - p_{ki} |$$

where D is the overlap between the two species, p_j is the frequency of occurrence of one concentration level and p_k is the frequency of occurrence of the same concentration level in the samples of the other species.

To assess the effects of food restriction and diet composition, we compared total mean concentrations of all detected bile acids calculated for each individual. Correlations were evaluated using Spearman's rank correlation coefficient.

3. Results

3.1 Qualitative differences

No species-specific differences could be found in the occurrence of the tested bile acids (Fig. 1). Bile acid bands corresponding to all standards (including cholic acid, not shown in Fig. 1) were detected in the samples of stone marten as well as in pine marten. In 80% of cases, the area of the corresponding cholic acid (CA) band was masked by pigments and lipid material included in the samples. Hence this bile acid was subsequently not considered. Besides, there was a great intraspecific variability between the patterns of bile acid contents in different faecal samples.

3.2 Quantitative differences

There were only slight differences in the frequency of occurrence of detected bile acid concentration levels between both species (Fig.1). When comparing the bile acid patterns of both species, we found high levels of inter-specific overlap for all detected bile acids with values of 79.5% (CAME), 82.4% (CDOCA), 82.4% (DOCA), 91.2% (DHCA) and 79.4% (LCA).

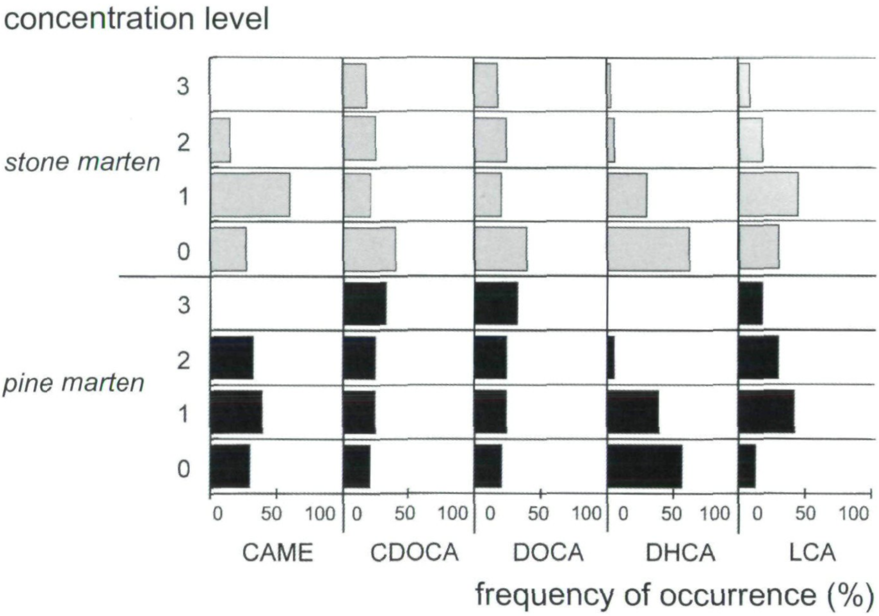


Fig. 1: The frequency of different bile acid concentration levels (0 = absent, 1 = low, 2 = medium, 3 = high) in faeces of captive stone martens (n = 34) and pine martens (n = 34).

3.2 Effects of food restriction

The quantity of ingested food affected the faecal bile acid concentration in both species. The average faecal bile acid concentration of all individuals decreased from *day 1* with normal diet (chicken or fish) to *day 2* (following day) when no food was available (Fig. 2).

3.3 Effects of diet composition

Within both species, the concentration of bile acids also depended on the dietary composition. The faecal bile acid concentration following a period of pure meat diet was higher than after feeding with a mixture of meat, fruit and cereal

(Fig. 3). As shown in Fig. 4, we also found that the portion of faecal vegetarian remains was negatively correlated with the mean concentration of faecal bile acids in both wild stone martens.

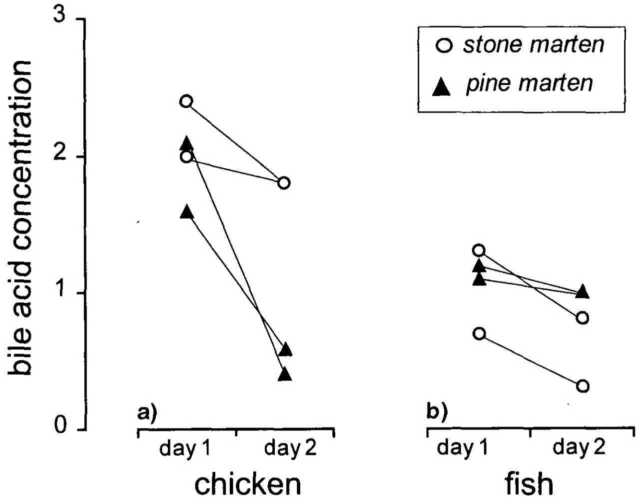
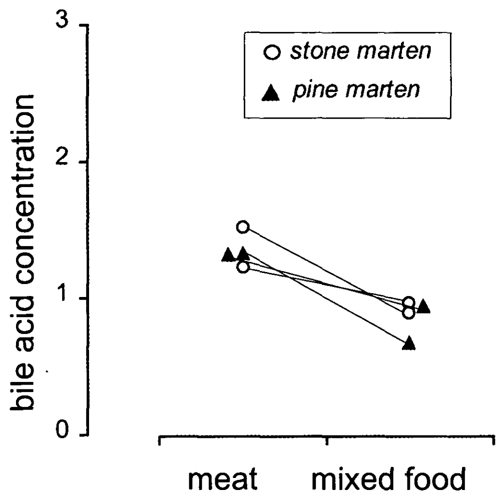


Fig.2: Comparison between the average faecal bile acid concentration in two captive stone martens and two captive pine martens on *day1* given a normal diet (chicken/fish) and the following day (*day2*) when no food was available.

Fig.3: Comparison between the average faecal bile acid concentration (n=6) of two captive stone martens and two captive pine martens after feeding with meat or mixed food (meat, fruit and cereal).



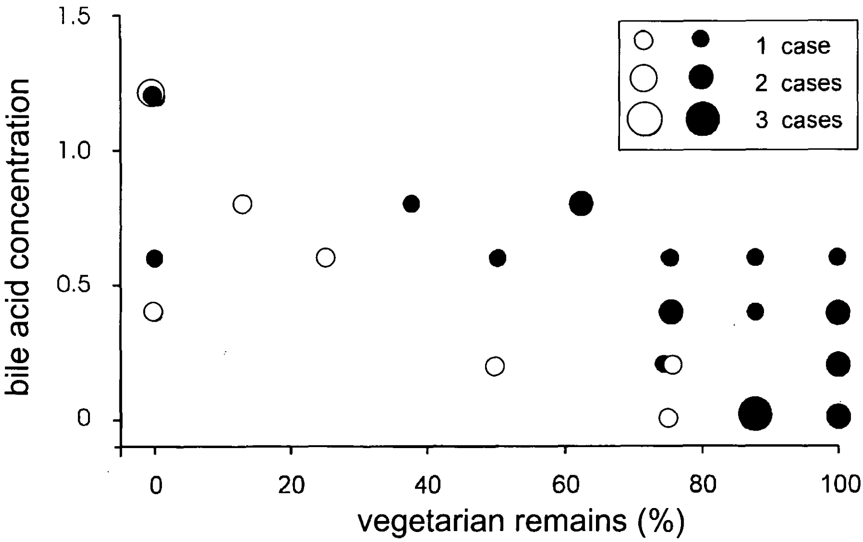


Fig.4: Correlations between the portion of vegetarian food remains (percentage of volume) and the average bile acid concentration in the faeces of two free-living stone martens (black circles: r_s=-0.657, P<0.001; white circles: r_s=-0.839, P<0.005).

4. Discussion

Our results show that the TLC method is not to use in the differentiation between stone marten and pine marten faeces. All standard bile acids were present in both species, thus an identification by the occurrence of different bile acids was impossible. As described by MAJOR et al. (1980) and JOHNSON et al. (1981, 1984), even concentration differences in some detected faecal bile acids can be sufficient to distinguish between species. Due to the high levels of overlap in the frequencies of the detected concentration levels in all tested bile acids, a determination of stone marten and pine marten faeces on the basis of this measure was also impracticable. The great intra-specific variability indicates that other factors influence faecal bile acid concentration.

One important factor is the quantity of food intake. Total bile acid concentration in all captive individuals of both species decreased after food restriction. Most of the primary bile acids (e.g. cholic acid, chenodeoxycholic acid) are re-absorbed in the ileum. Primary bile acids which are not absorbed come into contact with bacteria in the colon and are transformed into secondary bile acids and other products (HASLEWOOD 1967, LOEFFLER 1993). During a period of food shortage, digesta remains longer in the gut. Consequently primary bile acids can be absorbed at higher rates. Thus the conversion of bile acids by bacteria in the colon might be increased. Both processes may lead to a decrease in the total faecal bile acid concentration.

Another factor that affects faecal bile acid concentration is dietary composition. All captive individuals of both species showed lower faecal bile acid concentrations after being fed with an omnivorous diet than after a diet of pure meat. The negative correlation between the proportion of faecal vegetarian remains and the total faecal bile acid concentration in both wild stone martens also indicates lowering effects of vegetarian food. NAGENGAST et al. (1993) and QUINN & JACKMAN (1994) also found that the proportion of fibrous plant compounds in the diet lowers faecal bile acid concentration, particularly through an increase in stool mass. In addition, especially fatty compounds, that predominantly occur in animal prey stimulate the contraction of the gallbladder (ANDREWS 1979) which consequently leads to a higher concentration of bile acids in the faeces. Finally, co-ingested bile acids in the liver, gallbladder or in the gut of martens' prey may also affect the analysis of faecal bile acid content.

5. Conclusion

We did not find sufficient differences in quality and quantity of faecal bile acid content between the stone marten and the pine marten for a reliable species-specific determination. Food intake and dietary composition were important factors influencing the concentration of bile acids in both species. We presume that the analysis of faecal bile acids is not a useful tool for determination of faeces from wild congeneric carnivores with an omnivorous diet.

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