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# Comparison of three methods to evaluate wild boar diet

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**Abstract.** Wild boar diet composition highly reflects the management of the species as well as the level of its damaging effect. For this reason we tried to prove similarity and reliability of three methods of wild boar diet analysis to find out their suitability in practical use. Gastrointestinal tracts of 27 wild boar specimens were sampled, with the stomach and faecal contents of each individual being analysed and compared. Stomach and faeces analyses were done by identification of food items under microscope and measuring their quantity volumetrically. The third method, so called "veterinary", was the simplest one lying in the visual estimation of diet items percentage content diluted and spread in water on a tray. The similarity evaluation by qualitative and quantitative indices and additionally the generalised additive model confirmed that it is possible to identify all major food items which indicate the main diet strategy using all three analysis methods. All three tested methods were relevant in terms of basic features of quantitative and qualitative dietary assessment. The simple "veterinary" method, based on pure estimation, was proved to be suitable for field studies.

Key words: Sus scrofa, food composition, diet analysis, stomach, faeces

#### Introduction

Wild boar (*Sus scrofa*) is widely distributed ungulate. Population growth of this species, its broad damaging effects together with epidemiological problems require to be urgently addressed in many European countries (Schley & Roper 2003, Massei & Genov 2004, Barrios-Garcia & Ballari 2012). The food composition of this species is one of the main ecological questions related to its management (Ballari & Barrios-Garcia 2014). It reflects its food supply, level of damaging effect and influences its condition and reproduction (Herrero et al. 2006, Merta et al. 2014).

Studies on the diet composition of ungulate species are associated with the problem of obtaining a sufficient number of samples which are usually represented by alimentary tract contents. Concerning the wild boar, a collection of these samples is limited to the hunting season and depends on good cooperation with wildlife managers. A collection of faeces samples is not so limited, but the identification of diet items is more difficult compared to stomach contents. This problem was discussed by many

authors, as many studies on various ungulates and their gastrointestinal tract contents were done and compared (e.g. Homolka & Heroldová 1992).

No microscopic dietary study on the similarity of stomach and faeces contents of the same wild boar individuals has been carried out yet. One of the aims of our study was to conduct such a research. In addition, a simple "veterinary" visual estimation-based method was tested and its accuracy compared with the exact volumetric measurement of stomach and faeces contents. The purpose was to determine whether this method is sufficiently precise for the use in wildlife management. We presumed that the volumetric analysis of the stomach samples would give the most precise results.

#### **Material and Methods**

Wild boar individuals were shot during winter and their alimentary tracts were analysed. To cover higher diet variability, animals were collected at two different localities in the Czech Republic – a lowland forest stand (16 samples) and a highland forest stand (11 samples).

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In total, 27 gastrointestinal tracts were analysed, with stomach and faecal (rectum) contents of each animal being evaluated separately. As the fragmentation of food items in stomachs and faeces was extremely variable and we found big food fragments (whole earthworms, acorns, grasses, seeds, etc.) in both, their quantity was measured volumetrically.

## Stomach and faeces analyses

A commonly used volumetric method (e.g. Homolka & Heroldová 1992) was adapted. Contents of stomach and rectum of the same animal was firstly homogenized and separate samples of about 0.5 l in volume were taken into polyethylene flasks and kept in a freezer until further processing. Before the analysis, samples were thawed at room temperature and rinsed in water on a sieve (mesh size 0.5 mm), because of a frequent presence of mud. This mesh size was chosen to preserve small seeds which were morphologically differentiated and identified in terms of a plant taxon. The solid fraction retained on the sieve was analysed in detail. For exact analyses, individual samples of 25 ml were used. Samples were gradually transferred onto glass Petri dish, diluted in water and examined under a stereo microscope (a minimal magnification 10× was used, for tiny items, e.g. seeds, up to 40×). Diet items were separated into individual dishes. The identification was carried out according to their anatomical structure to the lowest taxon possible. Collected items were volumetrically measured using graduated cylinders of various volumes with a precision of 0.05 ml, after an excess of water was removed using an absorbent paper. The relative volume (%v) of each item was calculated.

# "Veterinary analysis"

For the visual rapid estimation (so called "veterinary" method), approximately the same volume of stomach contents (25 ml) was used. Samples were rinsed with water on a sieve (mesh size 0.5 mm) and analysed on a tray (preferably white for better visibility of the items, circa  $50 \times 40$  cm). Samples were evenly spread on the bottom of the tray in a thin layer of water (about 1 cm) preventing food particles from piling up one over another. The percentage cover of particular items was estimated based on visual assessment. These values were taken as the relative volume (%v). This estimation gives information about the dominant food components which can be used as a basic notion of the diet composition. This knowledge may be useful for hunters that can be informed about the use of supplementary food, among others, or for veterinary workers that can be notified of the causes of alimentary disorders or poisoning (Cellina 2008). Micro-histological recognition of food particles was based on plant morphology. Reference collection of plant samples and catalogues of food items were used for identification purposes. Due to the difficulty of identifying some plants and animals in terms of taxons, these were pooled into groups: for instance grasses, seeds and fruits (e.g. wild plant species seeds and mast), fruits of apple, pear or plum trees, other corns (e.g. wheat, barley or oat), invertebrates, vertebrates or roots.

#### Statistical evaluation

Trophic diversity was expressed by the Shannon-Weaver index (Shannon & Weaver 1949) based on the relative volume of each food item. Comparison of this index was done by modified t-test (Poole 1974). In addition, the equitability was calculated according to the formula  $J' = H'/\ln S$ , where H' is the Shannon-Weaver index and S is the total number of food components found in the sample. The permutation diversity test was used to verify the results. The qualitative similarity between the methods (all possible pairs of the methods were tested) was expressed by the Community Coefficient CC (syn. Sørensen index). The modified CC index for multiple-samples similarity measurement was calculated to evaluate the similarity of all three methods (Diserud & Ødegaard 2007). The Percentage Similarity PS (Quantitative Sørensen index) was used to express the quantitative similarity. Its adjusted version for multiple samples was also calculated to compare all three methods. Differences in individual food items were analysed using GAMLSS for zero-inflated beta distribution.

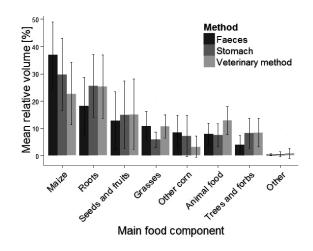


Fig. 1. Main food components assessed by three different methods (in % of volume).

**Table 1.** Diversity indices and diversity permutation test for three methods: S - Stomach, F - Faeces, V - "Veterinary" method (significant differences: \*p < 0.05; \*\*p < 0.01; \*\*\* p < 0.001).

	S	F	V	S : F	F : V	S : V
Number of items	20	19	16	0.53	**	***
Dominance_D	0.191	0.217	0.158	0.49	0.07	0.19
Simpson_1-D	0.809	0.789	0.866	0.49	0.37	0.16
Shannon-W H'	2.142	2.184	2.294	0.75	0.37	1.16
Evenness_e^H/S	0.426	0.467	0.620	0.60	0.07	*
Equitability_J	0.689	0.711	0.791	0.59	0.07	*

**Table 2.** Community coefficient (CC) and percentage similarity (PS).

	S : F	S:V	F : V	All
CC	0.97	0.89	0.86	0.95
PS	0.77	0.84	0.70	0.84

All statistical tests were executed in the PAST and R statistical software. All analyses were performed with a 5 % significance level (Anderson 2001).

# Results

We have identified 20 food items in total. All of them were discernible in stomach, while in faeces the silage was not identifiable. "Veterinary" method did not detect small seeds and fruits, invertebrates, moss and bark in any of the samples.

Diversity indices – Shannon-Weaver index H', Simpson index, and Dominance – for the wild boar diet composition obtained by various methods did not reveal any statistically significant differences. The Evenness and Equitability index J significantly differed between the stomach and "veterinary" method (Table 1).

For all individual index pairs, no significant difference in the Shannon-Weaver index H' was found (p < 0.05) (as per the modified t-test). The same applies for the modified Simpson index. The qualitative similarity (CC) and Percentage Similarity (PS) were considerably high (Table 2).

There was no statistical difference in the relative volume of 13 food items (out of 20) between the three methods. The relative volume of beetles in the "veterinary" method differed from that in the faeces contents. The "veterinary" method proved to be significantly inaccurate in the case of apples, larvae and earthworms.

As for main (dominant) food components (%v > 3), results show no significant differences in the relative volume between the tested methods (Fig. 1).

#### **Discussion**

All three methods (stomach, faeces and "veterinary") of the diet analysis proved to be precise enough to reflect the real wild boar diet as for main food components. The stomach volumetric analysis was the most precise, but time consuming. Faeces were analysed by the same method, as they were coarse and fibrous. Precision of the faeces analysis was influenced by the digestion process (Baubet et al. 2004). Both of these methods, analysed under microscope, are being employed mostly by experienced food ecologists with special laboratory equipment, but they are not suitable for direct field work.

Any study that attempts to infer a diet composition from analyses of stomach and faeces contents suffers from a number of problems. One drawback of these experiments is the fact that there is no constant digestibility rate for all food items. Fast digestion of soft tissues (4-5 hours) (Guerin et al. 2001) may result in underestimated volumes. Additionally, some food items stay longer in the intestine than others, which results in their accumulation. Grasses are usually excreted after 3-4 days in average. The proportion of woody plants may be underestimated, as in some cases wild boars chew shoots and roots, swallow the sap and starches, and reject woody tissues (Ballari & Barrios-Garcia 2014). The precision of the stomach analysis proved to be the highest as fragments are larger and easier to be determined.

Fournier-Chambrillon et al. (1996) and Baubet et al. (2004) considered both diet analysis methods (stomach- and faeces-based) adequate and comparable and used them in one feeding ecological study. In our study, high similarity of the stomach and faeces composition confirms this presumption.

The "veterinary" method does not discover small items observed only by a stereo microscope, e.g. small seeds, larvae or earthworms. Without a closer look several food items are easily interchangeable, e.g. an apple could be misinterpreted as a beet root. In the

case of less represented items, the absence could have been caused just by the sparse occurrence instead of the method failure. Considering the simplicity and practical usefulness of the "veterinary" method it is in fact a very interesting result. The method proved to be informative enough to reveal the influence of the use of supplementary food, which was found to be dominant in the diet, and have an effect on the wild boar overpopulation (Cellina 2008).

Even though the wild boar diet has been intensively studied during the last decades, a detailed local diet study could provide key information for the local wildlife management (Ballari & Barrios-Garcia 2014).

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