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AUTUMN DIET OF THE EUROPEAN HARE (*LEPUS EUROPAEUS*) IN AN ORCHARD IN THE NASZÁLY HILLS

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ABSTRACT
The European Hare (*Lepus europaeus*) is an important but declining game species in most of Europe and the availability of food is an important limiting factor. We investigated the Autumn diet of hares in a small orchard farm in Northern Hungary to determine what proportion of their food was composed of fruits and protein-rich fabaceous plants with the expectation that fabaceous plants would be more important in the diet. European Hare faecal pellets were collected on a single day, the entire study area was systematically searched for pellets. Additionally, reference samples were made of fruit and fabaceous plants collected on the study site on the same day. The diet was investigated using microhistological analysis of the epidermis fragments found in the European Hare pellets. The diet was separated into four categories: seeds, fruits, fabaceous plants and others. It was also investigated if the number of pellets taken from different droppings used for the analysis would have a significant impact on the results. The distribution was tested using the Chi^2 test and was found to be insignificant, the results suggest for a study area of such small size the number of pellets used to investigate the diet is not important. European hares did not consume high amounts of fruit and fabaceous plants, each only accounted for less than five percent of their diet and fabaceous plants were not consumed in a significantly higher amount compared to fruits on this farm area.

Keywords: microhistology, faecal pellet, fruit, Fabaceae

INTRODUCTION

The European Hare, *Lepus europaeus*, is a species that is native to much of Europe and parts of Asia (GREEN ET AL., 2013) and is a popular game species in Europe (SANGIULIANO ET AL., 2016). Despite this the European hare population has had a declining trend in Europe since the 1960s (SMITH ET AL., 2005). While there are various reasons for this decline such as climate change, increased numbers of predators, diseases, and human impacts (REICHLIN ET AL., 2006) it is believed that the main reason is due to agricultural intensification which has resulted in the decrease of farmland biodiversity and the decline of many other species (SMITH ET AL., 2004). European hares prefer heterogeneous habitats where there is a varied food supply and sufficient shelter throughout the year (KATONA ET AL., 2010).

The availability of food is one of the main limiting factors for the density of small mammals such as the members of the Leporidae family (KATONA ET AL., 2004). Hares have a broad diet that can be highly variable and include mainly grasses, shrubs and forbs with the proportions varying depending on the habitat and important factors such as the availability of food, weather conditions, predators, and interspecific competition as well as the season (PUIG ET AL., 2016).

There are several methods for investigating the diet compositions of European hare however a widely used indirect technique for analysis is the micro-histological faecal pellet or stomach content analysis based on the identification of epidermis fragments (KATONA AND ALTBACKER, 2002). Although this method has flaws and often can only roughly categorize the plants parts of the stomach contents (REICHLIN ET AL., 2006) in this case

since only a few specific plant species were the focus of this paper it was deemed appropriate to use.

While there are many studies focused on the diet of European hares, there are no studies focusing on the importance of fruit to the autumn diet of hare. The aim of this paper was to investigate the proportion of fruit in the diet of European hares living in an orchard area as well as investigate if they preferred high protein fabaceous plants such as white clover and alfalfa. It is expected that hare will consume fruit, based on other studies apples and plums are likely to be consumed however fruits will not take up a significant part of the hare's diet, while fabaceous plants are expected to be consumed by hare and are expected to take up a significant portion of the diet.

Additionally, the number of faecal samples needed to give an accurate analysis of the diet was investigated. For this, slides were prepared from 1 faecal pellet, 5 faecal pellets, 10 faecal pellets and a homogenous mixture composed of pellets from each pellet group found on the study site. The number of pellets used in the analysis has been shown to have a significant impact on the results, however, due to the small size of the study area significant difference is not expected.

MATERIALS AND METHODS

The study area was a small farm of 0.76 ha in Hungary located at the foot of the Naszály hills just outside of the town Vác in Pest County (*visible on Figure 1*). The vegetation of the study area is predominantly wild grass species, weeds, herbaceous plants including fabaceous species and various cultivated fruit trees and shrubs and very small, forest patch. The area around the study site is dominated by small sized farms with similar vegetation, forest patches and larger forested areas (mainly oak and beech species), roads and the mine areas which includes a clay, sandstone and limestone mine. The area is frequented by hare despite various disturbances (active maintenance of the orchard, lawn mowing and the occasional presence of dogs), adult and leverets have been spotted on the study site in previous years. Based on past observations a few different individuals are suspected of accessing the area.

Figure 1: Google maps satellite image of the study area (outlined in black)

Data Collection

The faecal pellets were collected on a single day, on November 13th in 2021. 22 different pellet groups, called droppings, were collected. As the study area was small the entire farm was searched for European hare pellets. Droppings were collected into individual bags. Several areas had a large number of pellets concentrated in one area, as it was not possible to distinguish different pellets near each other, about 0.5 meter, was considered as one pellet group and was collected into one bag, old pellets (dry, yellow, falling apart) were not collected. The diet composition of hare was determined by micro-histological faeces analysis following the methods from KATONA AND ALTBACKER (2002) and KATONA ET AL. (2004). Reference slides were made of the available fruits and fabaceous species collected from the study site on the same day as the pellets were collected. The species of fruit collected included the common medlar (*Mespilus germanica*), rose hip (*Rosa canina L.*), apple (*Malus domestica*), plum (*Prunus domestica*), seaberry (*Hippophae rhamnoides*), blackberry (*Rubus fruticosus*), grape (*Vitis vinifera*), and quince apple (*Cydonia oblonga*). Additionally, reference slides were made for two fabaceous species: white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*). The reference slides were prepared according to the description given by Mátrai and Katona (2004).

The microhistological analysis was performed on 1 pellet, 5 pellets, 10 pellets and 22 pellets all sampled from different dropping groups. The pellet(s) were placed in a Petri dish and homogenized with a small amount of distilled water. Out of the mixed subsample 1g was placed into a test tube and boiled with 2g of 20% nitric acid for 1.5 minutes. After that, the contents were poured onto a watch glass and using a scapple the fragments were pushed to the slides of the watch glass. The epidermis fragments were placed on the slide with two drops of 87% glycerine and one drop of 0.2% Toluidine-Blue. 5-6 slides were prepared for analysis. These procedures were repeated with the 4 sample sizes. 100 epidermis fragments on the slides were identified five times for each sample size under 40X magnification grouped according to 4 different categories: seed, fruit, Fabaceae and other with the help of the reference pictures.

Data Analysis

For each sample size the average results were calculated for each of the different plant categories. After this the data was analysed using the Chi^2 test to see if the diet composition attained using different faecal sample sizes differed significantly from each other or not using the averaged values. Additionally, each set (1,5,10 and 22) was tested for uniform distribution using the average values to see if there was a significant difference in the consumption of the different plant categories. Finally, the Chi^2 test was used again to see if there was a significant difference in the proportion of fruit and fabaceous plants in the diet for each of the different sample sizes.

RESULTS

Other plant species took up the largest proportion of the diet of European hares, in all sample sizes they accounted for 80% or more of their diet. Other plants were observed to take up the largest proportion of the diet with a sample size of 10 pellets where they took up 90.6% of the diet and the lowest amount when one pellet was used where they took up 80.4% of the diet. Seeds, fruit and fabaceous plants took up less than 20% of the diet combined for all the averaged values of the sample sizes. The highest proportion of seeds consumed was found when a sample size of one pellet was used where it accounted for

13.8% of their diet. The lowest proportion of seeds in the diet was 4.2% and was observed with a sample size of 22 pellets. The highest proportion of fruit consumed was 4.4% and was observed with the sample size of 22 pellets, while the lowest value was 1.6% and was observed with the sample size of 5 pellets. For Fabaceae consumption, the largest proportion was 4% found in the sample size of 22 pellets and the smallest was 2.4% observed in the sample size of 10 pellets. The diet composition for the four different pellet sample sizes can be compared on *Figure 2* where the averaged values are shown on the bar graph.

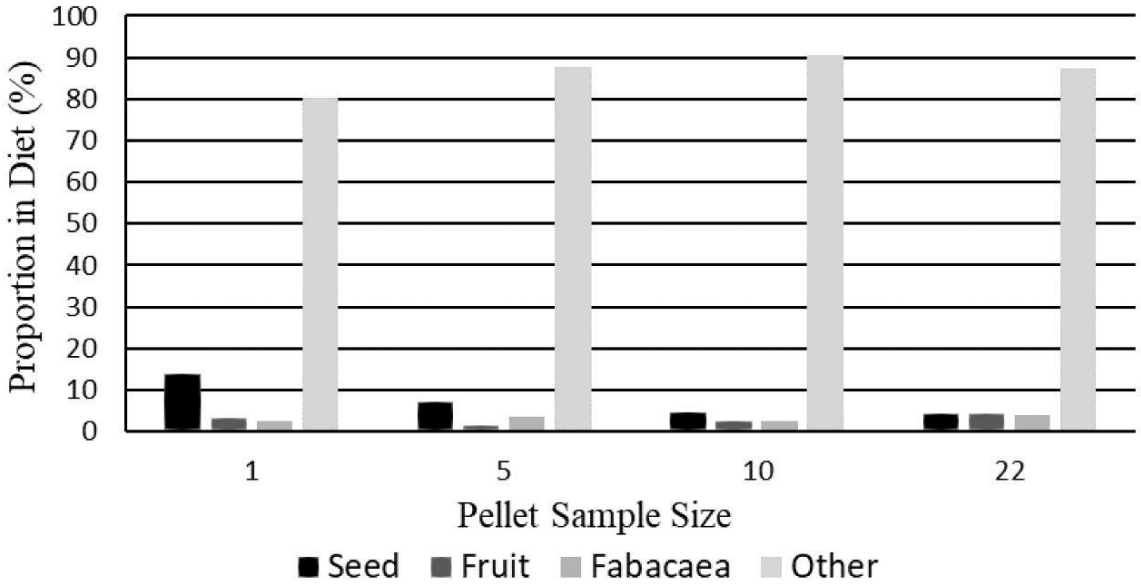

Figure 2: The composition of autumn diet of the European hare for the different pellet sample sizes in the study site.

Although the diet composition varied for the different sample sizes the Chi^2 test results showed that there was no significant difference between the proportion of plant species in the diet for the different sample sizes ($Chi^2 = 10.60$, df = 9, P = 0.308).
Additionally, none of the different sample sizes had a uniform distribution, in all cases, the Chi^2 value showed a significant deviation from that distribution (Ch^2 1: 166.86, Ch^2 5: 210.99, Ch^2 10: 229.64, Ch^2 22: 207.67, df= 3, P < 0.001). *Figure 3* compares the values of the observed and expected values.
There was no significant difference between the proportion of fruit and fabaceous plants in the diet, for each sample the Chi^2 value was insignificant (Ch^2 1: 0.06, Ch^2 5: 0.66, Ch^2 10: 0, Ch^2 22: 0.02, df = 1, P = 0.05).

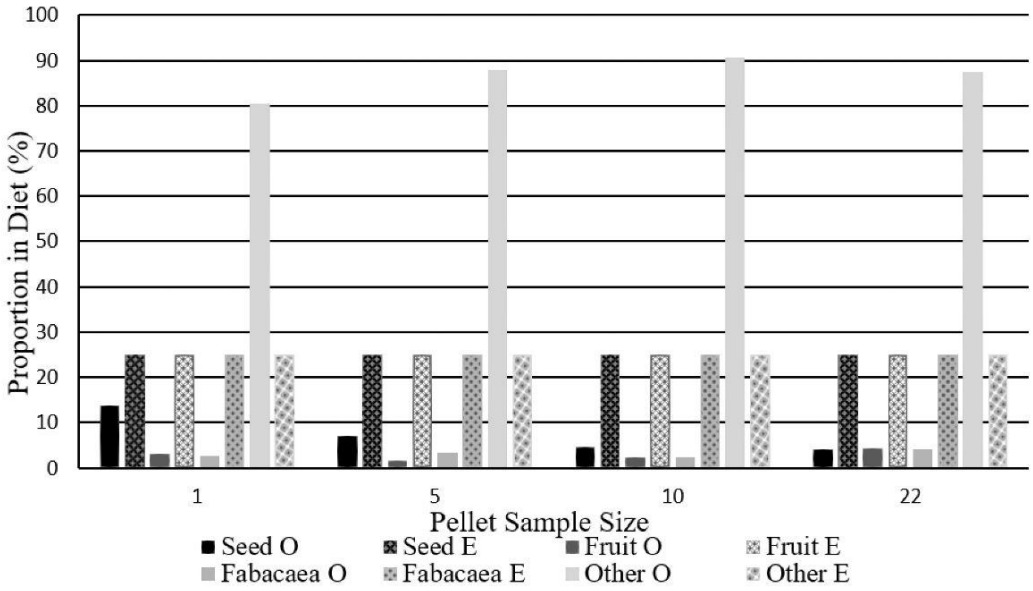

Figure 3: The expected uniform distribution compared with the observed values. (E=expected, O=observed)

DISCUSSION

Despite the availability of various fruits hare do not seem to prefer them but will consume them in a small amount. Additionally, although fabaceous plants are rich in protein (SCHAI-BRAUN ET AL., 2015) hare also did not consume them in high amounts. In comparison seeds were slightly more important.

Although previous studies have found a significant difference between the contents of pellets from different dropping and suggest using a minimum of 10 dropping for the micro-histological analysis (KATONA AND ALTBACKER, 2002) this study found no statistically significant difference. This is likely due to the small study size and the probability that droppings were from only a few individuals.

The study method itself, the micro-histological analysis, also has some limitations as it is difficult to accurately identify specific species of plants, and thus broader categories are often used with this methodology including in this study. Studying the stomach content of the hare may have given more accurate results, studies have suggested that if a large number of seeds and fruits are consumed it may be a preferable method (SOKOS ET AL., 2015; GREEN ET AL., 2013). However, faecal analysis is non-invasive and was more readily available for this study.

It would also be worth investigating how the diet changes with the seasons and if that would impact the proportions of the different categories seen in the diet. The diet of hare changes according to the season (GREEN ET AL., 2013) therefore it would be interesting to see if the proportion of fruit would change as well or if it would remain just a small part of the diet.

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