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„Detection of diet components in faecal samples of the Common hamster *Cricetus cricetus*“

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Abstract

Common hamsters are food-storing hibernators and show high individual variation in hibernation performance, which may be related to the quality of food hoards. This study tried to validate a new non-invasive method in the diet analysis of the common hamster based on mass spectrometry analysis of plant-specific proteins in faecal samples. The experiment was carried out under controlled conditions and the hamsters were fed a managed amount of predetermined foods. Faecal samples were collected, proteins extracted with Urea and put through mass spectrometry. An algorithm called ProtMAX groups all spectral counts of peptide-features with the same mass to charge ratio (*m/z*) at a certain retention time, where those peptide-features can then be analysed without further sequence information. This data-independent approach is called Mass Accuracy Precursor Alignment (MAPA). After subsequent filtering for specific peptide-features, an intrinsic peptide-mass-fingerprint (PMF) for each plant type fed to the animals remained. Furthermore peptide-features present in all samples were filtered, as potential hamster-specific PMFs, which could be used for species identification. With these PMFs we successfully created plant-specific biomarkers, which can be used to identify consumed plant species and allow to reconstruct a hamster´s diet. The application of this method allows to differentiate between stored and consumed diet components, which makes it possible to compare individual feeding- and overwintering-strategies. Using this technique for the analysis of faeces from wild hamsters could improve our knowledge on their handling of food-stores during winter, which subsequently enables a more successful hamster-friendly management and resettling of captively bred individuals into the wild.

Keywords Common hamster, MAPA, peptide analysis, faeces, diet, conservation

Introduction

Biology and Ecology

The common hamster *Cricetus cricetus* is a rodent from the family Cricetidae and is distributed throughout central Europe and parts of Russia (Figure 1). Formerly a steppe inhabitant it has become a synanthropic species living in agricultural and urban areas (Weinhold 2009). Hamsters are solitary, ground-dwelling animals, living in underground burrow systems, but must come to the surface to cache food and mates. These burrow systems consist of several chambers connected through tunnels, at least two exits, and are changed multiple times throughout the season. They can also be distinguished between summer and winter burrows; the winter burrows are greater in depth (Grulich 1981). Observations demonstrated that males change burrows more often than females and cover a wider range in search for mating partners (Kayser & Stubbe 2003). *C. cricetus* is a facultative hibernator and, depending on the geographical range, has an active season between March/April to September/October (Franceschini & Millesi 2008, Weinhold & Kayser 2006). Contrary to fat-storing hibernators, such as the European ground squirrel *Spermophilus citellus* or the marmot *Marmota marmota* (Humphries *et al.* 2003), common hamsters are food-storing hibernators, which means they have to rely on food reserves accumulated during the active season (Florant & Healy 2012, Humphries *et al.* 2003, Sheriff *et al.* 2013). This makes the quality of the food available even more important for survival during the winter.

Fig.1: Distribution of the European hamster (*Cricetus cricetus*) throughout central Europe
Source: IUCN

Diet

Living primarily on a plant-based diet, they are also known to consume invertebrates or even small vertebrates, such as birds, amphibians, and other mammals. Tissier *et al.* (2019) showed that hamsters living in agricultural areas predominantly consume weeds and wild plants, whereas hamsters in urban areas have diets dominated by tree flowers, fruits and seeds. Also present in their diet to varying degrees, depending on the availability, are vegetables and aromatic plants, fodder crops and leguminous plants, oilseeds, cereal and root crops. A variety of foods is an essential component in producing a healthy diet for hamsters. A deficiency in nutrients can lead to serious effects in reproduction, e.g. maize monocultures can cause maternal infanticide due to a lack of vitamin B3 (Tissier *et al.* 2016). The hamster's diet is dependent on many factors including seasonal changes, availability, harvesting trends, sex, and age. (Hufnagl *et al.* 2011). A 2011 study from Hufnagel et al. reported that 80% of pouch contents consisted of green plant parts throughout the season, with berries and rhizomes only present after May and seeds being more present before July. Later, in the season the importance of storable food items, such as crops and nuts becomes more significant, as they are more appropriate for hoarding in the burrow because of their high energy content (Roswag *et al.* 2018). Food stores are not only important for the winter period but also for the time just before emergence in spring, when they terminate hibernation but stay inside their burrows for several days or even weeks to prepare for the upcoming active season by, e.g., activating their reproductive system (Barnes *et al.* 1986, Michener 1992). Although females usually appear above-ground in spring later than males (Millesi *et al.* 2004, Siutz *et al.* 2016, Weinhold & Kayser 2006), they were found to shorten the post-hibernation euthermy, emerge and start reproduction earlier when supplemented with high quality food before winter (Siutz *et al.* 2018). This highlights the importance of food stores for both overwintering and reproduction in the forthcoming season. In general females are almost only seen caching above ground and are therefore assumed to feed inside their burrows, whereas males have been observed feeding regularly above ground (Siutz *et al.* 2012, Figure 2). These observations have led to the assumption that females accumulate larger food stores during the season than males. Furthermore, on average, males change burrows more frequently than females, in search of mating partners, and hence, it would be impractical to keep large food stores (Kayser & Stubbe 2003). On the other hand, females are more philopatric and use fewer burrows during the season. Food stores can therefore be used as energy source also during gestation and lactation period. (Siutz *et al.* 2012). During the lactation allowing a female to reduce

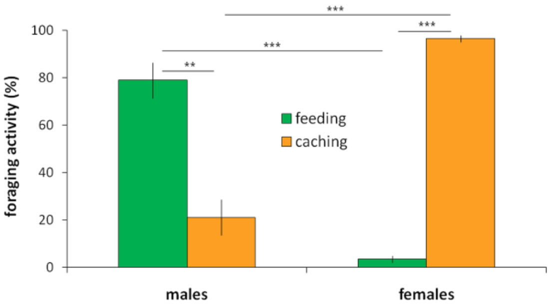


Fig.2: Proportion of foraging activities feeding and food caching above-ground in adult males (n = 14) and females (n = 24) (means ± SE) Siutz et al. 2012 pp. 160

surface activity and, hence, exposure to predators. Due to these sex difference in foraging strategies, however, observing females' consumption behaviours is problematic and information on which food items are consumed immediately or stored is lacking. This makes studying the feeding behaviour of females increasingly interesting.

Threats

As already mentioned, the common hamster is a synurbist, inhabiting croplands, orchards and other rural and urban areas. In the past, they were highly abundant in these areas and were even considered a pest and killed by hundreds (Feoktistova *et al.* 2013). This led to a massive decline in the hamster populations throughout Europe. Recent threats include excessive agriculture including pesticides, loss of cover due to early harvests and monocultures, as well as habitat loss and fragmentation (Albert 2013, Surov *et al.* 2016, Tissier *et al.* 2019, Weinhold & Kayser 2006).

Diet Analysis

To render conservational measures to a species needs, gathering as much information as possible is important. A vital component to study is an animal’s diet as it determines suitable habitats. For animals like the common hamster, who party feed inside burrows, more advanced techniques have to be applied. In contrary to earlier, very invasive studies, where stomach contents were analysed (Górecki *et al.* 1975), observing *C. cricetus* can give a good picture of how much and what they collect. A study carried out by Hufnagel *et al.* in 2011 showed that animals caught in Tomahawk live traps empty their cheek pouches inside the traps, which made it possible to get empirical and more detailed data on what they collect. Information on what they eat when inside the burrow is harder to obtain. Collecting and analysing faecal samples is probably one of the most widely utilized non-invasive method when studying the herbivore diet. Traditional microhistological examination of microscopic plant fragments in faeces, is still one of the most common techniques and gives a good overall picture of the animal’s diet (Stewart 1967). However, one of the draw backs is that identification can become impossible when some of the consumed matter was completely digested or was too fragmented (Fitzgerald & Waddington 1979). Furthermore, if identification is possible most of the time only the family level is able to be reported. A different approach in faecal analysis is DNA barcoding, a method mainly known in studying carnivore diet, using the Cytocrome Oxydase I gene from the mitochondrial DNA. The studies using this approach were carried out, e.g., in Norway on bears and voles, using a standardized DNA region, the P6-loop of the chloroplast trnL (UAA) intron of the chloroplasts. The results showed 50-75 % of the plants being identified to the genus level, some even to the level of subspecies (Soininen *et al.* 2009, Valentini *et al.* 2009). In case of the common hamster this method is not that suitable, since a fair amount of the diet consists of nuts, cereals, seeds and roots which do not contain chloroplasts in great quantity or at all.

Another study with the aim to be minimally invasive in the study of the diet of *C. cricetus* was carried out by Roswag *et al* (2018), where stable isotope ratios of animal tissue (hair) and consumed food were analysed. However, the ratios of the same food source varied too greatly between study sites and different food sources showed similar stable isotope ratios, undermining the results of this approach. Plant wax components, such as plant cuticular alkanes (predominantly n-alkanes), have been used to estimate the intake and diet composition in big herbivores, originally for ruminants (Dove & Mayes 1996) it was later also established for non-ruminants (Kuntz *et al.* 2006). For this method markers containing a known quantity of artificial even chain alkanes are fed to the animals to compare the external and the internal markers in faecal samples. Due to the need of an external marker, the suitability of this method in the study of the hamster diet is questionable, since hamsters are food-hoarders, and one might not know if the markers were eaten or not.

Proteomics-MAPA

The aim of this study was to validate a new method using Proteomics to analyse diet components in the common hamster*.* Proteomics focuses on the large-scale study of proteome, proteins and peptides as well as their expression and interactions (Blackstock & Weir 1999). With Peptide Mass Fingerprinting pre-digested, unknown proteins are put through mass spectrometry and fragmented into smaller peptides whose mass or mass to charge ratio (*m/z*) can be accurately measured (Clauser *et al.* 1999). The Mass Accuracy Precursor Alignment is a novel approach to shotgun proteomic analyses. It was developed by Hoehenwarter *et al.* in 2008 and defines the proteome by a single parameter instead of the previously needed two or even the primary structure of a peptide. Using the accurately measured peptide mass to charge ratio (*m/z*) allows us to compare MS/MS spectral analyses without previous peptide identification. This quantitative shotgun analysis method has since been used in the field of plant Proteomics e.g. for the identification of tryptic peptides in different protein isoforms in potato tubers *Solanum tuberosum*, where several cultivar-specific markers could be assigned to a specific cultivar and showed that protein isoforms, determine the different cultivar phenotypes (Hoehenwarter *et al.* 2008; Hoehenwarter *et al.* 2011). In this study we tried to apply this method to research questions related to Zoology and Behavioural Biology by testing if plant parts, that have passed an animal's digestive system are still suitable for protein analyses and possible plant-diet identification. The idea was to assign intrinsic peptide-masse-fingerprints (PMFs) to given food sources, which then can be used to identify the plants in question. For this approach, hamsters were kept under laboratory conditions and were fed different kinds foods, both perishable and storable, and determined plant specific peptides were extracted from the faecal samples.

Material & Methods

Animals and Housing Conditions

The study was conducted on five adult common hamsters, obtained from a breeding colony in Heidelberg, Germany in 2017. One was female and four were males. The animals were kept at the University of Vienna in constant condition chambers. Each hamster was housed individually in a plastic cage (99x51.5x36cm) and provided with an artificial burrow system consisting of three chambers connected through plastic tunnels (Figure 3). The chambers could be opened from the top, which allowed easier access to food stores and collection of faeces. The hamsters received standard rodent food pellets and had access to water *ad libitum*. Photoperiod lengths were adapted to natural daylight periods and temperature was gradually increased from 17 to 20°C. Experiments started with their natural emergence in spring (mid-April).

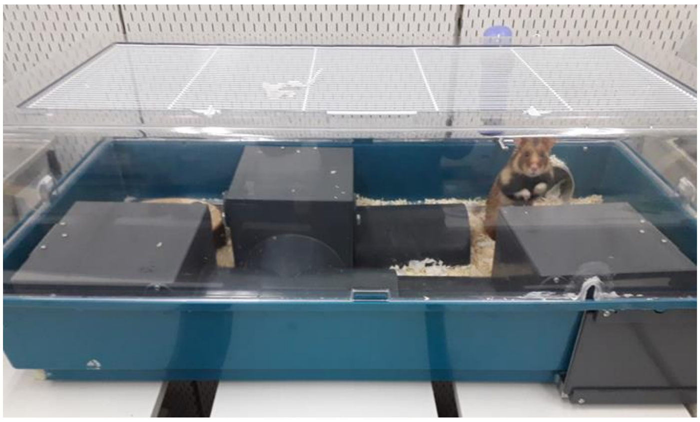


Fig.3: Housing cage of the hamster at the University with 3 plastic chambers connected through tunnel.

Experimental Procedure

Preceding the experiment, cages were thoroughly cleaned to remove previous food stores. For the experiment, the hamsters were fed with five different types of plants. Those were wheat (*Triticum aestivum ssp.)*, corn (*Zea mays),* sunflower seed (*Helianthus annus)* and dandelion (*Taraxacum sp.)*. Initially, the hamsters were given 150g of each type of food separately over a period of about two days. Finally, 100g of all five types of food were given together as a mix over a period of five days. The animals were weighed before, during and after the experiment to control for potential body mass changes.

Sample Collection

The faecal samples were collected every 1.5 hours over a period of two days, between 9 a.m. and 2 p.m.. Freshly dispensed faeces were labelled specifically. Immediately after collection, samples were stored at -80°C until analyses. From 114 collected samples, 13 were put through mass spectrometry: 3 corn, 3 wheat (analysed twice), 2 sunflower seeds, one dandelion and 4 of the mix. The experiments lasted between 2 and five days. Two wheat trials with the animals 9-17 and 5-17 took place in September 2019, whereas all others were carried out between April June of the same year.

Protein Extraction

The samples were ground with liquid nitrogen, in a mortar on ice, and 60 mg were weighed into an Eppendorf tube, filled with 1 ml 80% Acetone, carefully mixed and then centrifuged (10000g, 10 min, 4°C). The acetone was put in a falcon tube, where all the supernatants were collected and the pellet was again washed with 1 ml 80% Acetone, homogenized in a glass homogenizer, ultrasonicated for 5 minutes and then again centrifuged (10000 g, 10 min, 4°C). The pellet was then extracted in 1 ml 80% Methanol and incubated at 95°C for 30 minutes. After the sample was cooled down to room temperature it was centrifuged (20000 g, 10 min, 4°C), pellet was re-extracted with 1 ml of 80% ethanol and incubated at 95°C for 30 minutes. After cooling down and centrifugation (20000 g, 10 min, 4°C), the pellet was solubilized with 200 µl of 8 M urea buffer in 50 mM Hepes, pH 7.8 and left at room temperature for five minutes before centrifugation (10000 g, 10 min, 4°C). Supernatant, containing the proteins, was precipitated using 1 ml acetone + 0,5% ß-mercaptoethanol at -20°C overnight. The pellet was resuspended in 200 µl urea buffer and the protein concentration was determined by the dye-binding assay (Bradford, 1976) using bovine serum albumin as standard. For each sample the amount containing between 10-20 µg of protein was calculated and adjusted to a final concentration of 20 µg per 200 µl with urea buffer. Immobilized Trypsin (Promega) was added for protein digestion (1/20) and incubated at 30°C overnight. In the next step the digest was quenched, adding 2 µl of pure formic acid to the 200 µl and centrifuged (10,000 g, 10 min, 4°C). The peptides were desalted on C_{18} stage tips (Pierce Thermo Scientific, USA). The tips were activated with 50% acetonitrile/50% dH_2O, equilibrated with 100 µl 0,1% formic acid and then two times 100 µl of the sample were loaded. After washing with 100 µl of 0,1% formic acid, the samples were eluted two times with 100 µl 100% acetonitrile and evaporation in a centrifugal vacuum system (Speedvac) and stored at -20°C until further analysis.

LC – MS/MS

Prior to MS analyses, sampels were solved in 2% acetonitrile, 0.5% formic acid. Per sample 1 µg was loaded onto a 15 cm × 50 µm column (PepMap®RSLC, Thermo scientific,2 µm particle size) and separated during a 90 min gradient (2-80% Acetonitril) with a flow rate of 300 nL min−1 onto a reversed phase (C18 column, Thermo) subsequently coupled to the mass spectrometer. Mass spectrometry measurements were performed on an LTQ-Orbitrap Elite (Thermo Fisher Scientific; Bremen, Germany) in data dependent mode. FT full scans were done with a maximum ion load (AGC) of 2 x 106, a resolution of 30,000 and a maximum injection time of 2000 ms. ITMS/MS scans were performed with an AGC of 1 x 104 and a maximum injection time of 50 ms. Collision induced dissociation (CID) was carried out with a normalized collision energy of 35%. Rejection of single or unassigned charges and monoisotopic precursor selection and dynamic exclusion of 15 s and charge state screening were enabled (Hoehenwarter *et al*. 2011). The rapid label-free shotgun approach was applied (Hoehenwarther *et al.* 2008; Figure 4). Biological replicas are put through mass spectrometry. The Program ProtMAX puts every *m/z* value with its respective spectral count (SC) and retention time (Rt) into a spectral sampling matrix. Some kind of sorting process, either by hand, like in this study and/or other multivariate statistical procedures is then applied to reduce the volume of the data and make in open for *de novo* interpretation.

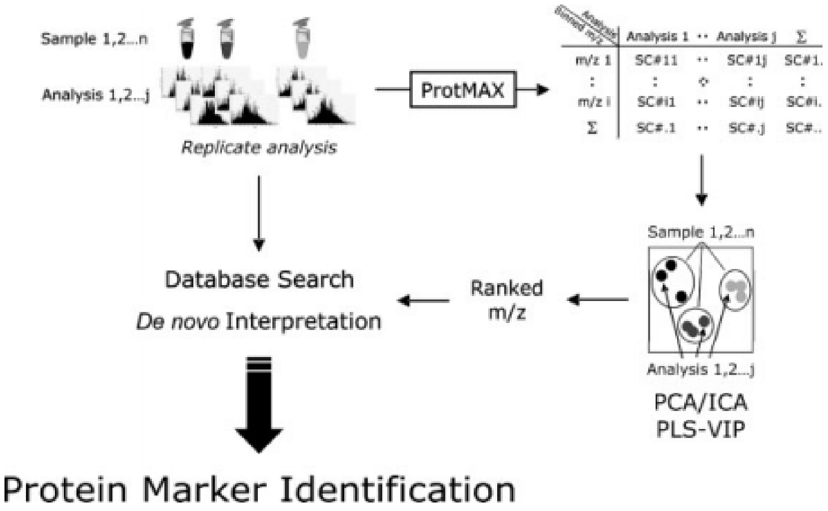


Fig.4: Workflow of the rapid label-free shotgun proteomics screening approach. Hoehenwarther *et al*. 2008 pp. 4217

Protein Data analyses

Unbiased approach

In order to analyse peptide features from faeces, we used the ProtMAX software tool (Egelhofer *et al.* 2013). Settings were default except for method: ion count and Quantification intensity.

LC-MS/MS – alignment analysis

The data from the mass spectrometry, the thermo .raw files were converted to mzXML format, the common file format for proteomics mass spectrometric data, with the ReAdW Software (MassMatrix MS Data File Conversion, v3.9, http://www.massmatrix.net/mm-cgi/downloads.py). The ProtMax software tool (https://mosys.univie.ac.at/resources/software/) helps with the automated alignment of precursors and will create a data matrix in a tab-delimited format that can be opened with Microsoft Excel (Egelhofer *et al.* 2013). With precursor *m/z* in the rows and mzXML file identifiers in the columns, the spectral counts of every mass to charge ratio was rounded to the second decimal. (Egelhofer *et al.* 2013). Peptides with masses occurring in every sample were separated as potential hamster faeces. For the specific peptide pattern of the various food sources, peptides not present in all samples but only in the specific food source were selected, separately (Figure 5).

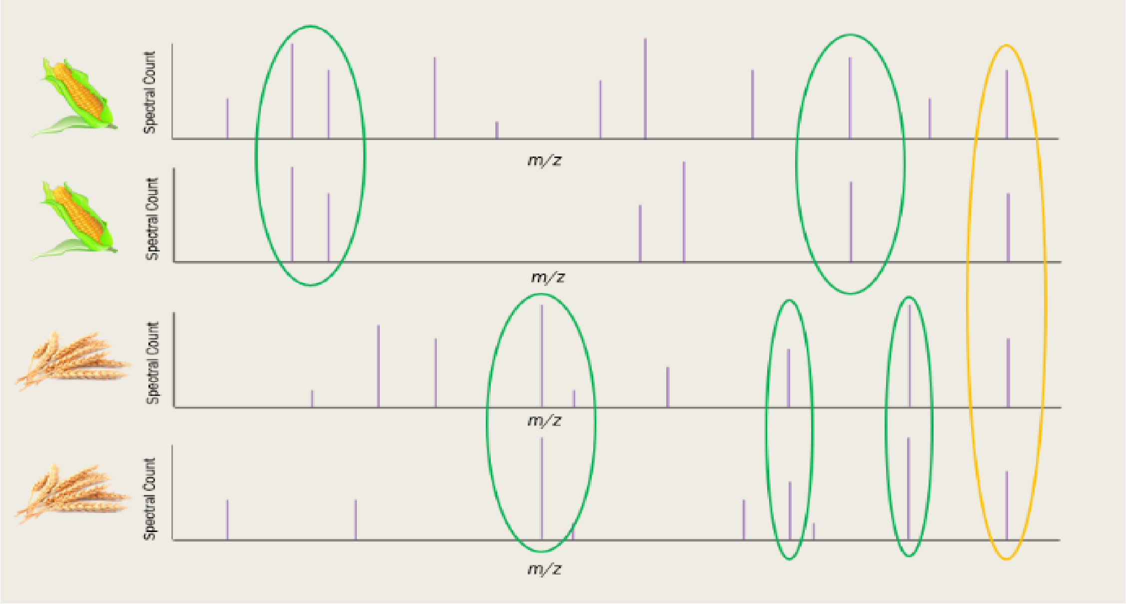


Fig.5: Simplified depiction of the sorting process of spectral counts for mass to charge ratios (*m/z*). Green circles: peptide masses only present in samples of specific food source; yellow circle: peptide masses present in all samples.

Results

Body Mass Change

The animals lost an average of 19.5g ± 11.54g (mean ± SD), of body mass during a trial but varying with given food source and duration. The two hamsters fed with sunflower seeds (IDs 9-17 & 6-17) had the smallest change with a loss of 1g and 3g, respectively, over a period of 3 days. Three individuals took part in three evaluations (9-17, 6-17 and 7-17), 8-17 participated in two and 5-17 in one (Figure 6). The female (5-17) had the biggest weight loss, feeding on wheat, of 39g over a period of 3 days.

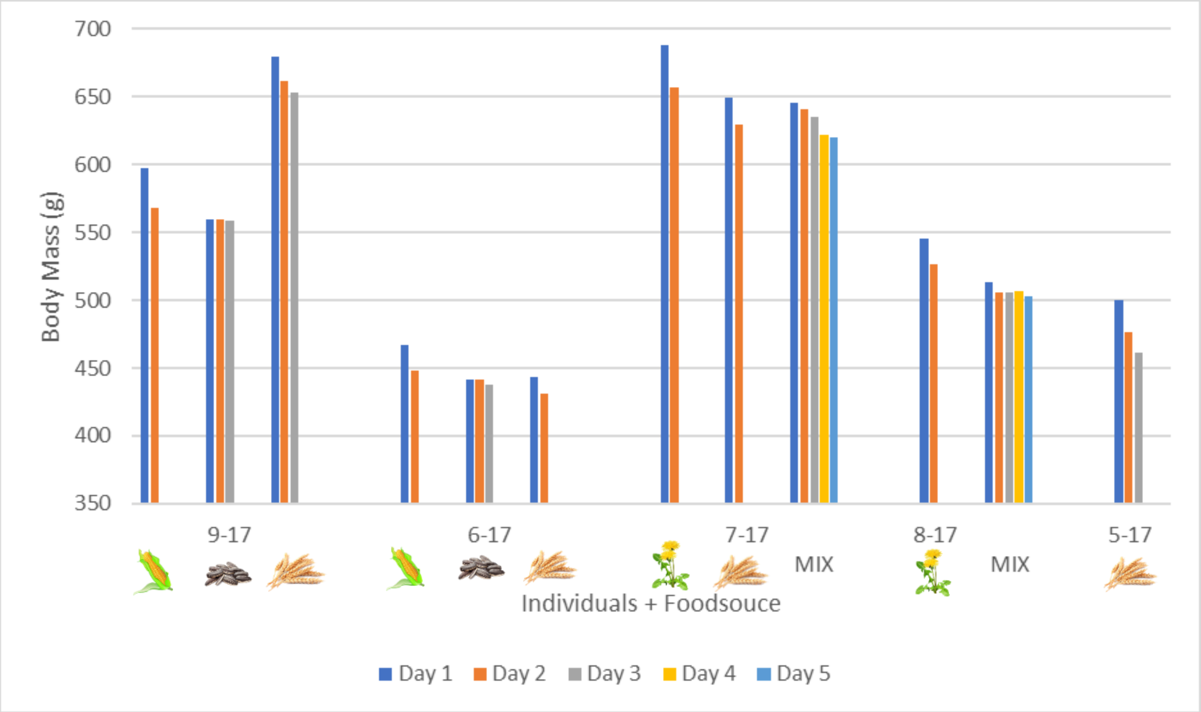


Fig.6: Changes in body mass of the hamsters (*Cricetus cricetus*), four males (IDs 9-17, 6-17, 7-17 and 8-17) and one female (ID 5-17), during 12 trials with a duration between 2 and 5 days. Given food sources where: corn (*Zea mays*), sunflower seed (*Helianthus aannus*), dandelion (*Taraxacum sp.*) and wheat (*Triticum aestivum ssp.*). Two wheat trials with the animals 9-17 and 5-17 took place in September 2019, whereas all others were carried out between April and June of the same year.

Peptide Mass Fingerprints

Four types of food were analysed using 13 samples. Table 1 shows the peptide mass features to charge ratios (*m/z*) at a certain retention time (RT) present in all faecal samples of the four individually/separately given food source as well as the mix. The corn (*Zea mays*) containing feces had 14 individual peptide mass features (PMF) present in all 3 biological replicates, sunflower seed (*Helianthus annuus*) 7 PMFs in 2 replicates, dandelion (*Taraxacum sp.*) 16 PMFs in the 1 and wheat (*Triticum aestivum ssp.*) 1 peptide in 3 replicates. PMFs present in all of the samples were 38 (Table 2). ProtMAX output files, including charge state and sum across all samples and for each sample spectral count of ion intensity counts, scan number of the most intense ion signal of the corresponding *m/z* value and retention time (Rt) of the most intense ion signal of the corresponding *m/z* value are available in the Appendix (Table 3; Table 4).

Table 1.: Peptide mass features (or mass to charge ratios (*m/z*)) with a certain retention time (Rt), extracted from faeces of the common hamster (*Cricetus cricetus*) and analyzed through MS/MS present in samples of different single food sources (corn, sunflower seed, dandelion and wheat) as well as in the Mix.

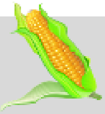
m/z										MIX		
	Retention Time											
466,27	70,2	70,2	69,8							70,6	70,5	70,3
586,79	56,4	56,5	56							56,9	56,8	55,2
589,31	56,9	56	57							57,3	57,4	57,1
602,81	70,7	70,3	70,4							71	71,2	70,5
681,37	76,1	76,5	76,8							76,6	76,5	76,3
705,83	68,3	67,7	68,4							68,7	68,7	68,4
773,42	85,8	86,2	85,6							86,2	86,2	85,8
778,84	106,8	107,6	107,8							107,7	107	106,9
811,49	116,3	116,1	117,1							116,5	116,5	116,3
877,53	120,8	120,6	121,5							120,8	120,9	120,8
878,99	73	73,1	74							73,3	73,3	73,1
905,46	107,4	107,6	108							107,7	107,9	107,5
910,8	106,3	106,7	107,3							107	106,8	106,7
965,43	110,7	111,2	111,7							111,4	111,4	110,6
688,76				104,3	104,5					104,3	104,1	104
697,85				93,6	94,8					94,3	94,1	93,6
748,37				96,3	95,7					96,4	96,2	95,9
817,31				92,5	93,4					91,8	92,2	92,1
828,3				92,3	91,4					92	92	91,7
838,81				103,7	103,9					103,6	103,4	103,2
929,4				92,7	93,1					92,2	92,3	92,1
442,21						145,3				144,8	145,6	145,8
546,27						43				42,5	43,1	43
564,78						96,7				95,9	95,8	95,4
628,85						8,7				8,8	9,2	9,7
660,83						98,3				98,2	98,3	97,9
684,32						93,2				92,8	92,8	92,6
713,39						109,4				108,8	109,3	109,1
743,83						108				108	107,7	108
795,31						107,3				106,9	106,6	106,4
815,31						20				21	20	20,5
815,31						18,7				19,5	18,8	19,2
832,87						95,8				95,8	95,8	95,4
898,43						91,9				91,7	91,7	91,5
928,46						81,7				81,4	81,4	81,3
931,95						98,5				99,7	99,1	98,9
1170,56						100,4				100,1	99,8	99,6
829,95							94,4	94,1	94	94,8	95	94,4

Table 2.: Peptide mass features (or mass to charge ratios (*m/z*)) with a certain retention time (Rt) extracted from faeces of the common hamster (*Cricetus cricetus*) and analyzed through MS/MS present in all samples.

m/z										MIX			
	Retention Time												
350,98	126,6	126,5	127,2	126,5	126,4	126,5	126,4	127,2	126,4	127,3	126,6	126,7	127
350,98	125,5	124,3	124,5	124,4	125,4	124,7	125,3	124,9	124,7	124,8	124,7	125,6	124,9
362,22	93,8	93,5	93,7	93,6	93,8	93,7	93,6	93,6	93,7	93,6	93,7	93,7	93,5
362,7	95,4	95,6	96	96,1	96,2	96	96	96	96,1	95,7	95,8	95,8	95,6
377,72	95,8	96	96,2	96,3	96,5	96,3	96,2	96,2	96,2	96	96,2	96	95,8
379,24	93,4	93,6	93,7	93,6	93,9	93,7	93,6	93,6	93,7	93,7	93,9	93,7	93,7
384,23	95,8	96	96,3	96,3	96,5	96,3	96,3	96,3	96,3	96,6	96,2	96,2	96
406,24	98,2	98,4	98,8	98,8	99,1	98,8	98,8	98,8	98,8	98,4	98,6	98,5	98,3
423,27	98,1	98,3	97,9	98,8	99	98,8	98,7	98,8	98,8	98,4	98,5	98,4	98,2
436,77	100,1	100,2	101,1	101,1	101,3	101,1	101,5	101,1	101,1	100,3	100,4	100,4	100,1
458,78	103,6	102,5	103,3	103,2	103,9	103,7	103,2	103,2	103,3	103,3	104,2	103,1	103,6
523,77	104,1	103,8	103,8	105,5	104,7	104,7	104,8	103,9	104,3	103,8	103,9	103,7	103,9
539,76	105,7	105,8	106,9	107,3	107,7	107,3	107,6	106,7	107,3	105,9	106,1	106,1	106,2
547,3	96,3	96	96,3	97	96,7	95,6	96,5	95,9	96,5	95,9	95,7	95,7	95,6
552,3	101,7	101,6	101,6	101,9	102,6	102,5	102,4	101,8	102,6	101,6	101,9	101,8	101,9
552,3	99,2	99,3	99,1	98,5	100	99,9	99	99,1	99,6	99,4	99,6	99,6	99,4
552,8	105,6	105,2	105,9	105,8	105,8	105,5	107,1	106,1	106,6	105,2	106	105,5	105,1
559,28	94,6	94,4	93,6	93	93,2	92,9	94,5	93,8	93,1	92,6	94,6	93,9	92,8
559,78	98,5	97,4	96,6	98,3	97,8	98,2	97,6	97,2	97,3	98,5	97,6	97,5	97,2
560,34	110,1	110,3	111,1	110,9	111,1	111	111,1	111	111	109,8	110,5	110,4	110,1
568,78	108,1	108,2	109,4	108,9	107,9	108,1	108,1	108	109,3	109,9	108,4	108,4	109,7
628,86	5,4	5,5	4,6	5,2	5,1	5,2	4,6	5,1	4,6	5,4	5,4	5,5	5,7
723,43	121,1	121,1	122	121,9	121,8	121,9	122,4	121,4	121,8	121,8	121,6	121,2	120,6
736,44	120,9	120,9	121,7	121,4	121,6	121,5	121,5	121,9	121,4	121	120,9	120,7	120,6
736,83	112,5	113,2	114,3	114,2	114,3	114,2	114,3	114,2	114,3	113,3	113,4	113,4	113,1
744,96	120,9	120,8	121,9	121,3	121,4	121,9	121,9	122,1	121,7	121,6	121,2	120,5	120,5
746,35	91,3	91,3	92,6	91,7	91,4	90,9	90,9	92,4	90,8	91	90,7	92,6	91,3
764,83	5,5	5,7	4,8	4,9	4,7	5,3	4,8	4,9	4,7	5,7	5,6	5,2	5,4
767,46	120,8	120,7	121,8	121,3	121,7	121,5	121,8	121,6	121,6	120,9	121,2	121	121,1
780,47	121	120,5	121,5	122,1	121,8	121,9	121,6	122,2	121,5	121	121,4	121	120,9
789,48	120,7	121,2	121,8	121,6	121,6	121,4	121,6	121,6	121,5	120,5	121,2	121,9	120,5
810,99	121,3	121,2	121,9	121,3	121,6	121,9	121,6	121,7	121,2	121,2	121,1	121,2	121,3
855,51	120,4	120,8	121,5	122	122,2	121,5	121,9	121,7	122,1	120,9	121,4	120,7	120,7
857,9	99,9	99,6	99,6	101,1	100,3	100,3	100,4	99,5	100,3	99,6	100,1	100,1	101,1
885,43	115,6	115,8	114,5	115,1	115,6	115,8	115,7	114,6	115,4	115,7	115,9	114,4	115,6
901,42	98,8	98,6	98,3	98,3	99,1	99,3	99,2	98,3	99	98,4	98,7	99	98,4
1075,51	102,1	102,2	102,4	102,3	103,3	103,3	103,1	102,6	103,2	102,2	102,4	102,3	102,1
1161,04	99,6	100,1	99,7	100,6	100,3	100,3	100,2	99,8	100,2	99,7	99,9	99,7	99,5

Discussion

Feeding the hamsters under controlled conditions, collecting their faeces and sorting the peptide-features after protein extraction and MS analysis resulted in specific peptide-mass-fingerprints that may be used for further field sampling experiments to determine what plant the animal was feeding on and to learn more about their feeding habits in their hibernacula during hibernation. The goal is to collect the last faeces before immergence and the first ones after emergence. This way it can be roughly determined what has been eaten and what has been stored at the respective times of collection.

Establishment of intrinsic Peptide mass Fingerprints for the different diets

13 samples were sent through mass spectrometry analysis. Since it was a trial study, not all food sources contain the same sample replication sizes. For stronger qualitative results probably a greater and/or even number of samples would be necessary, but for the purpose of this initial experiment an unequal number of samples was sufficient. From the corn and wheat samples, three replicates were analysed in August 2019 and another one of each in July 2020, which shows that samples could be stored over a longer period of time at -80°C and still contain enough proteins for further analysis. The reason why males are overrepresented in this study is because females were part of a breeding program excluded from the study to avoid the risk of malnutrition due to single food sources. There might me a possibility that PMFs are biased towards males. In a second round of trials, a female was included since it took place after the breeding season. To validate a difference between males and females an equal number of both sexes with more trials and the same food source would be needed to get significant statistical data.

A first attempt was made to compare the spectral peaks to already existing plant databases, it turned out, however, that the proteins were already strongly digested. This also challenges protein identification, since an *in silico* digestion with no defined protease cleavage site was not possible to carry out (to less computation power). Nevertheless, we gained thousands of MS2 peptide-features which we used for the MAPA approach. This previous collection of peptide masses must be done for each relevant food source individually. When studying hamsters in the field a survey of available plants, as well as observations of collected plants can be done in advance and can be fed to hamsters under controlled conditions. This would enable to create and adjust peptide-feature databases for each study target specifically, and allow a direct comparison between available, collected and actually consumed food sources. Hamster are seen as food opportunists adapting their diet to the type of habitat, although not all habitats are necessarily equipped with the same nutritional value, reflecting their reproductive success (Tissier *et al.* 2017). In addition to the process peptide masse features occurring in all the samples were also sorted, possibly indicating to be hamster specific. This could be used to determine the presence of hamsters through faecal findings, especially in areas also inhabited by other ground-dwelling rodents e.g. European ground squirrels.

Advantages of Proteomics

Even though Proteomics is a lot more complex than Genomics, since the genome is more or less constant, whereas proteins differ from cell to cell and from time to time, it usually gives us a better understanding of an organism. The approach is also fitting for organisms with not fully sequenced genomes, which cannot be recognized or identified by genome/EST-based search algorithms (Hoehenwarter *et al.* 2008). Diet analyses, especially scatological ones, have already been using DNA sequences as biomarkers, although this was primarily done on predatorial animals (Deagle *et al.* 2005, Jarman *et al.* 2004). There have been studies applying the DNA barcoding method to herbivores, while they also showed restrictions due to the diet composition. DNA markers used for detection and identification are based on chloroplast DNA, therefore chloroplast-rich plant parts, like leaves and stems, might be overrepresented in comparison to roots and seeds (Soininen *et al.* 2009). As for this, DNA barcoding would prove to be considerately problematic in the hamster study, as they are known to make a lot of their intake, especially during winter, out of those plant parts (Górecki & Grygielska 1975). As we want to improve our knowledge on their winter diet, or consumption during hibernation, having some important nutritional sources underrepresented and others overshadowing the results would be very disadvantageous. Peptide mass features could provide as a more reliable and data independent approach for detection and identification of diet components. Although not in already digested samples, Proteomics has already been shown to be successful in the analysis of dietary elements. Dairy consumption in humans could be verified using ancient dental calculus dating 6 millennia back, using the same method, the liquid chromatography tandem mass spectrometry (LC-MS/MS), to identify the whey protein β-lactoglobulin (Bleasdale *et al.* 2021, Warinner *et al.* 2014). Another study documented dairy products mixed with cereals through LC-MS/MS peptide analyses in residue in ancient ceramic vessels, dating back approximately 8000 years. Not only are peptides in greater quantity present in plant foods than e.g. lipids, they also enable more detailed taxonomic identification (Hendy *et al.* 2018). This shows that proteomic shotgun approaches are consequently less impacted by protein degradation and can detect denatured as well as fragmented proteins, even after a very long period of time.

Body mass change

In addition to our main study question, changes in body masses were also recorded. This was primarily done to secure the animals health during the trials. The animals lost between 1 and 39 grams of body mass during a trial, depending on the food source and duration. During both trials where the animals were fed with sunflower seed, body mass almost did not change at all. As seen in a number of papers where hamsters were supplemented, sunflower seeds were often used because of their high fat content (Siutz *et al.* 2017, Siutz *et al.* 2018). In this study, however, this was just a notable observation and was not continued further for any statistical significance. Another notable difference that showed up, is that the hamster with the ID 9-17 took part in two trials during spring and another one in autumn where it started out with a much higher body mass. This could indicate the accumulation of body mass during the active season.

Food stores affect hibernation behaviour

As food storing hibernators the quantity and quality of the accumulated reserves is directly related to the hibernation performance and especially the duration and numbers of torpor bouts, (Siutz *et al.* 2017). Hibernation is a way to reduce energy and water loss during the winter season. The animals alternate between torpor bouts and arousals (Ruf & Geiser 2015). Duration of torpor expressions can differ between individuals and depends on internal energy reserves and external food stores (Siutz *et al.* 2017). Increased fat reserves lead to longer torpors, but they also come at a cost, as they are found to be associated with oxidative stress (Carey *et al.* 2000; Giroud *et al.* 2009), impaired memory loss (Millesi *et al.* 2001), immune depression (Franco *et al.* 2013; Prendergast *et al.* 2002), ischemia (Carey *et al.* 2003) and reduced synaptic efficacy (Strijkstra *et al.* 2003). This implies a cost-benefit trade-off between energy reserves and hibernation performance (Humphries *et al.* 2003). A study under controlled conditions showed that hamsters with a reliable food hoard in their hibernacula would postpone deep torpors to the end of winter. Furthermore, individuals provided with food stores with high fat content, like sunflower seeds, again reduced their time spent in deep torpor (Siutz *et al.* 2017). This shows once more how important not only the quantity but also the quality of the food hoard is. While males can be observed feeding above ground, females are almost always caching food above-ground and consuming it inside their burrows. This is also reflected in different overwintering strategies between males and females, where males accumulate more body fat during the active season, start hibernation earlier than females and also spent more time in torpor (Siutz *et al.* 2012; Siutz *et al.* 2016). Individuals provided with high quality supplementary foods before winter showed sex-specific differences in hibernation performance, in that males immerged later in autumn and reduced their time spent in torpor compared to unsupplemented males. Supplemented females emerge earlier in spring and start reproduction earlier (Siutz *et al.* 2018). Leftover high-quality food stores could allow less time spent caching food above-ground during gestation and lactation and increase the surviving chance of a female and her offspring. In free ranging hamsters, faecal samples collected immediately after emergence in spring could provide information on what types of food were recently consumed and, hence, are still left at the end of winter indicating if and how the animals ration their available food. Furthermore, faecal samples collected during the active season are also of interest, especially from females, to gain insight into nutritional needs during gestation and lactation periods. It can be assumed that perishable food is consumed first, but this has yet to be documented. Faecal samples from inside the burrow, however, are still hard to access, without opening and/or destroying the burrow but could nonetheless be obtained by, e.g., live-trapping. With this method we hope to find a new indirect way to study the animal's diet. To attain viable results, as previously mentioned, a combination of available food sources in the study area, collected items plus the faecal samples would be advantageous. The differentiation between perishable and non-perishable foods is important for food caching animals, especially hibernators, as well as the time handling collecting food in relation to eating it.

MAPA utilisation in conservation

The Common hamster (*Cricetus cricetus*) was already strictly protected by the Appendix IV of the Fauna-Flora Habitat Directive in all countries of the European Union, which prohibited the hunting, killing or the destruction of burrows. Until recently the species was considered of least concern (LC) by the IUCN, but as of July 2020 it was listed as critically endangered (CR) (Banaszek *et al.* 2020).

The decline of the species in the wild is due to a combination of many factors. Like for most animals, habitat loss and fragmentation play a major role. Hamsters in general have very high winter mortality rates, which might be related to disturbed food storing activities in autumn and insufficient availability of high-quality foods (Albert 2013, Francescini-Zink & Millesi 2008). Most of the reasons for a population decrease are directly or indirectly linked to agricultural management, for example early harvests lead to a lack of cover and adequate winter supplies accompanied by a reduction of cover significantly increasing the risk of predation (Kayser *et al.* 2003). Others include the heavy use of pesticides and monocultures (Tissier *et al.* 2019). Over the last years increasing efforts have been made to try to establish a hamster-friendly agricultural management, like planting of winter crops or leaving unharvested corridors between fields (Losinger & Pöter 2008). Knowledge of collected and consumed food in relation to its availability, enables to improve the nutritional value of food sources in suitable habitats which could, ultimately, increase the reproductive success. The results of this and following studies using MAPA could provide us with greater knowledge to apply crucial measures in hamster-friendly agricultural management to render their needs and re-create favourable habitats.

The MAPA approach as a non-invasive and indirect method for detection of food components could provide a new time efficient and affordable tool in the field of diet analysis, especially for animals who are difficult to observe. Given the early stage of the method in this field, future trials should validate more/different plant species, could include other rodents and on the long hand eventually herbivores in general.

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Appendix

Tab.3: Already sorted ProtMAX output file for the food specific sorting process. Charge state and sum across all samples. For each sample (34, 35, 36, 44, 45, 55a, 61, 64, 111, 77, 93 & 94) spectral count of ion intensity counts, scan number of the most intense ion signal of the corresponding m/z value and retention time (Rt) of the most intense ion signal of the corresponding mass to charge (*m/z*) value. Corn *Zea mays* (yellow), Sunflower seed *Helianthus annuus* (grey), Dandelion *Taraxacum sp*. (green), Wheat *Triticum aestivum ssp.* (red), MIX (blue).

			Corn								
			I			II			III		
m/z	Charge state	Count [all]	34 [Count]	34 [Scan]	34 [Rt]	35 [Count]	35 [Scan]	35 [Rt]	36 [Count]	36 [Scan]	36 [Rt]
877,53	2	9	1	19059	120,8	1	25902	120,6	1	41625	121,5
811,49	2	11	2	18353	116,3	2	25135	116,1	1	40854	117,1
965,43	2	11	1	17164	110,7	2	23961	111,2	2	38886	111,7
905,46	3	8	1	16570	107,4	1	23169	107,6	1	37551	108
778,84	2	10	1	16448	106,8	2	23167	107,6	1	37448	107,8
910,8	3	11	1	16351	106,3	2	22944	106,7	2	37278	107,3
773,42	2	9	1	12810	85,8	2	17847	86,2	1	29375	85,6
681,37	2	9	1	11214	76,1	2	14892	76,5	1	26112	76,8
878,99	2	7	1	10726	73	1	13777	73,1	2	25056	74
602,81	2	11	3	10399	70,7	1	12926	70,3	1	23676	70,4
466,27	2	8	1	10310	70,2	2	12884	70,2	1	23455	69,8
705,83	2	6	1	10009	68,3	1	12114	67,7	1	22918	68,4
589,31	2	9	1	8311	56,9	2	8864	56	2	18570	57
586,79	2	7	2	8232	56,4	1	8982	56,5	1	18173	56
688,76	2	5	0	0	0	0	0	0	0	0	0
838,81	2	9	0	0	0	0	0	0	0	0	0
748,37	2	6	0	0	0	0	0	0	0	0	0
697,85	2	8	0	0	0	0	0	0	0	0	0
929,4	2	7	0	0	0	0	0	0	0	0	0
817,31	2	17	0	0	0	0	0	0	0	0	0
828,3	2	8	0	0	0	0	0	0	0	0	0
442,21	2	5	0	0	0	0	0	0	0	0	0
713,39	2	13	0	0	0	0	0	0	0	0	0
743,83	2	5	0	0	0	0	0	0	0	0	0
795,31	2	4	0	0	0	0	0	0	0	0	0
1170,56	2	6	0	0	0	0	0	0	0	0	0
931,95	2	6	0	0	0	0	0	0	0	0	0
660,83	2	5	0	0	0	0	0	0	0	0	0
564,78	2	5	0	0	0	0	0	0	0	0	0
832,87	2	6	0	0	0	0	0	0	0	0	0
684,32	2	5	0	0	0	0	0	0	0	0	0
898,43	2	6	0	0	0	0	0	0	0	0	0
928,46	2	4	0	0	0	0	0	0	0	0	0
546,27	3	4	0	0	0	0	0	0	0	0	0
815,31	2	13	0	0	0	0	0	0	0	0	0
815,31	2	8	0	0	0	0	0	0	0	0	0
628,85	3	8	0	0	0	0	0	0	0	0	0
829,95	2	13	0	0	0	0	0	0	0	0	0

	Sunflower seed						Dandelion			Wheat		
	I			II			I			I		
m/z	44 [Count]	44 [Scan]	44 [Rt]	45 [Count]	45 [Scan]	45 [Rt]	55a [Count]	55a [Scan]	55a [Rt]	61 [Count]	61 [Scan]	61 [Rt]
877,53	0	0	0	0	0	0	0	0	0	0	0	0
811,49	0	0	0	0	0	0	0	0	0	0	0	0
965,43	0	0	0	0	0	0	0	0	0	0	0	0
905,46	0	0	0	0	0	0	0	0	0	0	0	0
778,84	0	0	0	0	0	0	0	0	0	0	0	0
910,8	0	0	0	0	0	0	0	0	0	0	0	0
773,42	0	0	0	0	0	0	0	0	0	0	0	0
681,37	0	0	0	0	0	0	0	0	0	0	0	0
878,99	0	0	0	0	0	0	0	0	0	0	0	0
602,81	0	0	0	0	0	0	0	0	0	0	0	0
466,27	0	0	0	0	0	0	0	0	0	0	0	0
705,83	0	0	0	0	0	0	0	0	0	0	0	0
589,31	0	0	0	0	0	0	0	0	0	0	0	0
586,79	0	0	0	0	0	0	0	0	0	0	0	0
688,76	1	23028	104,3	1	25462	104,5	0	0	0	0	0	0
838,81	2	22822	103,7	1	25245	103,9	0	0	0	0	0	0
748,37	1	20220	96,3	1	22315	95,7	0	0	0	0	0	0
697,85	1	19279	93,6	2	21994	94,8	0	0	0	0	0	0
929,4	1	18950	92,7	1	21386	93,1	0	0	0	0	0	0
817,31	3	18874	92,5	4	21493	93,4	0	0	0	0	0	0
828,3	1	18794	92,3	1	20785	91,4	0	0	0	0	0	0
442,21	0	0	0	0	0	0	1	28114	145,3	0	0	0
713,39	0	0	0	0	0	0	1	21704	109,4	0	0	0
743,83	0	0	0	0	0	0	1	21362	108	0	0	0
795,31	0	0	0	0	0	0	1	21178	107,3	0	0	0
1170,56	0	0	0	0	0	0	1	19026	100,4	0	0	0
931,95	0	0	0	0	0	0	1	18524	98,5	0	0	0
660,83	0	0	0	0	0	0	1	18457	98,3	0	0	0
564,78	0	0	0	0	0	0	1	18018	96,7	0	0	0
832,87	0	0	0	0	0	0	2	17766	95,8	0	0	0
684,32	0	0	0	0	0	0	1	17060	93,2	0	0	0
898,43	0	0	0	0	0	0	2	16734	91,9	0	0	0
928,46	0	0	0	0	0	0	1	14310	81,7	0	0	0
546,27	0	0	0	0	0	0	1	7790	43	0	0	0
815,31	0	0	0	0	0	0	1	3937	20	0	0	0
815,31	0	0	0	0	0	0	1	3646	18,7	0	0	0
628,85	0	0	0	0	0	0	2	1380	8,7	0	0	0
829,95	0	0	0	0	0	0	0	0	0	1	20149	94,4

	Wheat											
	Irep			II			Iirep			III		
m/z	61rep [Count	61rep [Scan]	61rep [Rt]	64 [Count]	64 [Scan]	64 [Rt]	64rep [Count	64rep [Scan]	64rep [Rt]	111. [Count]	111. [Scan]	111. [Rt]
877,53	0	0	0	0	0	0	0	0	0	0	0	0
811,49	0	0	0	0	0	0	0	0	0	0	0	0
965,43	0	0	0	0	0	0	0	0	0	0	0	0
905,46	0	0	0	0	0	0	0	0	0	0	0	0
778,84	0	0	0	0	0	0	0	0	0	0	0	0
910,8	0	0	0	0	0	0	0	0	0	0	0	0
773,42	0	0	0	0	0	0	0	0	0	0	0	0
681,37	0	0	0	0	0	0	0	0	0	0	0	0
878,99	0	0	0	0	0	0	0	0	0	0	0	0
602,81	0	0	0	0	0	0	0	0	0	0	0	0
466,27	0	0	0	0	0	0	0	0	0	0	0	0
705,83	0	0	0	0	0	0	0	0	0	0	0	0
589,31	0	0	0	0	0	0	0	0	0	0	0	0
586,79	0	0	0	0	0	0	0	0	0	0	0	0
688,76	0	0	0	0	0	0	0	0	0	0	0	0
838,81	0	0	0	0	0	0	0	0	0	0	0	0
748,37	0	0	0	0	0	0	0	0	0	0	0	0
697,85	0	0	0	0	0	0	0	0	0	0	0	0
929,4	0	0	0	0	0	0	0	0	0	0	0	0
817,31	0	0	0	0	0	0	0	0	0	0	0	0
828,3	0	0	0	0	0	0	0	0	0	0	0	0
442,21	0	0	0	0	0	0	0	0	0	0	0	0
713,39	0	0	0	0	0	0	0	0	0	0	0	0
743,83	0	0	0	0	0	0	0	0	0	0	0	0
795,31	0	0	0	0	0	0	0	0	0	0	0	0
1170,56	0	0	0	0	0	0	0	0	0	0	0	0
931,95	0	0	0	0	0	0	0	0	0	0	0	0
660,83	0	0	0	0	0	0	0	0	0	0	0	0
564,78	0	0	0	0	0	0	0	0	0	0	0	0
832,87	0	0	0	0	0	0	0	0	0	0	0	0
684,32	0	0	0	0	0	0	0	0	0	0	0	0
898,43	0	0	0	0	0	0	0	0	0	0	0	0
928,46	0	0	0	0	0	0	0	0	0	0	0	0
546,27	0	0	0	0	0	0	0	0	0	0	0	0
815,31	0	0	0	0	0	0	0	0	0	0	0	0
815,31	0	0	0	0	0	0	0	0	0	0	0	0
628,85	0	0	0	0	0	0	0	0	0	0	0	0
829,95	1	20149	94,4	1	21159	94,1	1	21159	94,1	3	30102	94

	MIX								
	I			II			III		
m/z	77. [Count]	77. [Scan]	77. [Rt]	93. [Count]	93. [Scan]	93. [Rt]	94. [Count]	94. [Scan]	94. [Rt]
877,53	1	24015	120,8	2	21760	120,9	2	21610	120,8
811,49	2	23321	116,5	1	21016	116,5	1	20844	116,3
965,43	2	22040	111,4	2	19902	111,4	1	19634	110,6
905,46	1	21237	107,7	2	19189	107,9	1	19031	107,5
778,84	3	21219	107,7	1	18987	107	1	18901	106,9
910,8	3	21028	107	2	18950	106,8	1	18871	106,7
773,42	2	16082	86,2	1	14540	86,2	1	14628	85,8
681,37	3	13716	76,6	1	12604	76,5	1	12685	76,3
878,99	1	12886	73,3	1	11926	73,3	1	12049	73,1
602,81	3	12412	71	1	11577	71,2	2	11614	70,5
466,27	1	12329	70,6	1	11457	70,5	1	11571	70,3
705,83	1	11932	68,7	1	11143	68,7	1	11213	68,4
589,31	1	10038	57,3	1	9298	57,4	1	9427	57,1
586,79	1	9982	56,9	1	9222	56,8	1	9154	55,2
688,76	1	20473	104,3	1	18377	104,1	1	18305	104
838,81	3	20298	103,6	1	18203	103,4	1	18139	103,2
748,37	1	18606	96,4	1	16626	96,2	1	16670	95,9
697,85	3	18064	94,3	1	16194	94,1	1	16183	93,6
929,4	1	17573	92,2	2	15831	92,3	2	15903	92,1
817,31	1	17463	91,8	4	15827	92,2	4	15894	92,1
828,3	1	17497	92	3	15752	92	2	15810	91,7
442,21	2	27329	144,8	1	25118	145,6	1	24952	145,8
713,39	4	21481	108,8	3	19472	109,3	2	19346	109,1
743,83	1	21306	108	1	19138	107,7	2	19117	108
795,31	1	21007	106,9	1	18899	106,6	1	18806	106,4
1170,56	1	19467	100,1	1	17411	99,8	1	17417	99,6
931,95	2	19340	99,7	1	17260	99,1	1	17276	98,9
660,83	1	18994	98,2	1	17059	98,3	1	17058	97,9
564,78	1	18483	95,9	1	16535	95,8	1	16546	95,4
832,87	1	18478	95,8	2	16544	95,8	1	16550	95,4
684,32	1	17700	92,8	1	15943	92,8	1	15992	92,6
898,43	1	17437	91,7	1	15687	91,7	1	15769	91,5
928,46	1	14870	81,4	1	13551	81,4	1	13695	81,3
546,27	1	8024	42,5	1	7340	43,1	1	7503	43
815,31	2	4753	21	4	3894	20	3	4234	20,5
815,31	2	4416	19,5	2	3641	18,8	2	3949	19,2
628,85	1	1745	8,8	1	1821	9,2	2	1741	9,7
829,95	3	18186	94,8	1	16369	95	1	16344	94,4

Tab.4: Already sorted ProtMAX output file for the hamster specific sorting process. Charge state and sum across all samples. For each sample (34, 35, 36, 44, 45, 55a, 61, 64, 111, 76, 77, 93 & 94) spectral count of ion intensity counts, scan number of the most intense ion signal of the corresponding mass to charge ratio (*m/z*) value and retention time (Rt) of the most intense ion signal of the corresponding *m/z* value. Corn *Zea mays* (yellow), Sunflower seed *Helianthus annuus* (grey), Dandelion *Taraxacum sp.* (green), Wheat *Triticum aestivum ssp.* (red), MIX (blue).

			Corn								
			I			II			III		
m/z	Charge state	Count [all]	34. [Count]	34. [Scan]	34. [Rt]	35. [Count]	35. [Scan]	35. [Rt]	36. [Count]	36. [Scan]	36. [Rt]
350,98	3	29	2	20041	126,6	2	26945	126,5	2	42710	127,2
350,98	3	47	4	19905	125,5	4	26661	124,3	4	42281	124,5
810,99	2	22	1	19190	121,3	2	26059	121,2	2	41757	121,9
767,46	2	33	4	19045	120,8	3	25927	120,7	2	41710	121,8
780,47	2	31	3	19119	121	3	25889	120,5	2	41626	121,5
855,51	2	35	3	18947	120,4	2	25954	120,8	2	41635	121,5
736,44	2	22	2	19087	120,9	2	25972	120,9	1	41688	121,7
723,43	2	33	2	19128	121,1	2	26040	121,1	3	41762	122
789,48	2	24	2	19037	120,7	1	26081	121,2	1	41700	121,8
744,96	2	28	3	19088	120,9	2	25959	120,8	2	41735	121,9
885,43	2	31	2	18114	115,6	1	25001	115,8	2	39891	114,5
736,83	2	26	2	17495	112,5	3	24427	113,2	1	39819	114,3
560,34	2	28	3	17050	110,1	3	23765	110,3	1	38651	111,1
568,78	2	41	2	16691	108,1	2	23282	108,2	1	38030	109,4
539,76	2	40	1	16237	105,7	1	22744	105,8	4	37119	106,9
552,8	2	47	2	16214	105,6	1	22628	105,2	1	36757	105,9
523,77	2	37	1	15949	104,1	1	22353	103,8	1	36020	103,8
458,78	2	44	2	15862	103,6	3	22043	102,5	4	35811	103,3
1075,51	2	15	1	15562	102,1	1	21959	102,2	1	35513	102,4
552,3	2	31	2	15490	101,7	1	21830	101,6	3	35198	101,6
857,9	2	47	4	15179	99,9	2	21323	99,6	1	34470	99,6
436,77	2	42	1	15213	100,1	2	21522	100,2	1	35015	101,1
1161,04	2	29	1	15114	99,6	4	21478	100,1	1	34505	99,7
552,3	2	32	1	15056	99,2	2	21267	99,3	1	34273	99,1
901,42	2	53	4	14985	98,8	2	21096	98,6	3	33985	98,3
406,24	2	15	1	14866	98,2	1	21035	98,4	1	34192	98,8
423,27	2	20	1	14856	98,1	1	21021	98,3	2	33863	97,9
559,78	2	56	4	14934	98,5	1	20816	97,4	1	33373	96,6
384,23	2	23	2	14484	95,8	1	20487	96	1	33266	96,3
377,72	2	24	1	14470	95,8	1	20481	96	1	33249	96,2
547,3	2	53	3	14552	96,3	4	20473	96	3	33256	96,3
362,7	2	17	1	14415	95,4	1	20370	95,6	1	33171	96
379,24	2	18	1	14097	93,4	2	19881	93,6	1	32321	93,7
362,22	2	26	2	14159	93,8	2	19847	93,5	1	32320	93,7
559,28	2	35	2	14276	94,6	2	20051	94,4	2	32301	93,6
746,35	2	44	2	13742	91,3	3	19277	91,3	2	31924	92,6
628,86	3	29	2	717	5,4	3	697	5,5	1	694	4,6
764,83	3	23	2	736	5,5	2	729	5,7	1	731	4,8

	Sunflower seed						Dandelion			Wheat		
	I			II			I			I		
m/z	44. [Count]	44. [Scan]	44. [Rt]	45. [Count]	45. [Scan]	45. [Rt]	55a. [Count]	55a. [Scan]	55a. [Rt]	61. [Count]	61. [Scan]	61. [Rt]
350,98	1	28669	126,5	2	31415	126,4	2	25389	126,5	2	29270	126,4
350,98	3	28398	124,4	1	31270	125,4	3	25124	124,7	1	29135	125,3
810,99	2	27860	121,3	2	30525	121,6	1	24632	121,9	1	28531	121,6
767,46	2	27855	121,3	1	30568	121,7	1	24553	121,5	1	28576	121,8
780,47	1	28027	122,1	2	30586	121,8	1	24643	121,9	2	28542	121,6
855,51	1	28017	122	2	30690	122,2	2	24548	121,5	2	28596	121,9
736,44	2	27872	121,4	1	30539	121,6	2	24545	121,5	1	28513	121,5
723,43	2	27989	121,9	3	30592	121,8	2	24648	121,9	1	28710	122,4
789,48	1	27915	121,6	1	30535	121,6	1	24520	121,4	1	28544	121,6
744,96	2	27861	121,3	2	30481	121,4	1	24626	121,9	1	28605	121,9
885,43	3	26563	115,1	3	29177	115,6	2	23335	115,8	3	27283	115,7
736,83	1	26260	114,2	1	28755	114,3	1	22914	114,2	1	26838	114,3
560,34	1	25241	110,9	2	27708	111,1	1	22077	111	3	25827	111,1
568,78	4	24569	108,9	2	26656	107,9	2	21397	108,1	5	24885	108,1
539,76	1	24030	107,3	3	26572	107,7	3	21175	107,3	5	24735	107,6
552,8	3	23527	105,8	3	25932	105,8	6	20655	105,5	5	24543	107,1
523,77	2	23429	105,5	4	25535	104,7	5	20387	104,7	4	23718	104,8
458,78	2	22639	103,2	2	25275	103,9	3	20078	103,7	3	23168	103,2
1075,51	1	22304	102,3	1	25064	103,3	1	19945	103,3	1	23146	103,1
552,3	3	22167	101,9	1	24804	102,6	2	19735	102,5	2	22885	102,4
857,9	1	21901	101,1	2	23951	100,3	7	18988	100,3	5	22219	100,4
436,77	4	21877	101,1	5	24317	101,3	6	19259	101,1	3	22609	101,5
1161,04	3	21702	100,6	3	23979	100,3	2	18999	100,3	2	22136	100,2
552,3	3	20972	98,5	1	23868	100	2	18865	99,9	3	21729	99
901,42	3	20927	98,3	4	23538	99,1	5	18728	99,3	6	21785	99,2
406,24	1	21092	98,8	1	23515	99,1	1	18599	98,8	1	21649	98,8
423,27	1	21080	98,8	1	23501	99	2	18587	98,8	1	21628	98,7
559,78	2	20906	98,3	2	23065	97,8	2	18441	98,2	8	21246	97,6
384,23	1	20228	96,3	1	22610	96,5	1	17926	96,3	2	20774	96,3
377,72	1	20200	96,3	2	22589	96,5	1	17908	96,3	5	20753	96,2
547,3	4	20456	97	5	22691	96,7	4	17714	95,6	5	20864	96,5
362,7	1	20126	96,1	1	22495	96,2	1	17835	96	1	20688	96
379,24	1	19269	93,6	1	21666	93,9	1	17180	93,7	1	19857	93,6
362,22	2	19268	93,6	1	21646	93,8	1	17179	93,7	2	19865	93,6
559,28	1	19047	93	3	21414	93,2	1	16990	92,9	2	20179	94,5
746,35	4	18591	91,7	2	20761	91,4	3	16516	90,9	6	18949	90,9
628,86	2	769	5,2	2	775	5,1	3	796	5,2	1	639	4,6
764,83	2	725	4,9	1	705	4,7	3	807	5,3	1	671	4,8

	Wheat											
	I rep			II			II rep			III		
m/z	61rep. [Cou	61rep. [Sca	61rep. [Rt]	64. [Count]	64. [Scan]	64. [Rt]	64rep. [Cou	64rep. [Scan	64rep. [Rt]	111. [Count]	111. [Scan]	111. [Rt]
350,98	2	29270	126,4	2	29331	127,2	2	29331	127,2	2	40218	126,4
350,98	1	29135	125,3	4	29028	124,9	4	29028	124,9	3	39946	124,7
810,99	1	28531	121,6	1	28502	121,7	1	28502	121,7	1	39253	121,2
767,46	1	28576	121,8	2	28494	121,6	2	28494	121,6	2	39334	121,6
780,47	2	28542	121,6	1	28625	122,2	1	28625	122,2	2	39326	121,5
855,51	2	28596	121,9	2	28501	121,7	2	28501	121,7	2	39464	122,1
736,44	1	28513	121,5	1	28563	121,9	1	28563	121,9	1	39308	121,4
723,43	1	28710	122,4	4	28434	121,4	4	28434	121,4	2	39405	121,8
789,48	1	28544	121,6	2	28497	121,6	2	28497	121,6	2	39332	121,5
744,96	1	28605	121,9	2	28594	122,1	2	28594	122,1	2	39361	121,7
885,43	3	27283	115,7	2	27117	114,6	2	27117	114,6	2	37912	115,4
736,83	1	26838	114,3	1	26997	114,2	1	26997	114,2	1	37503	114,3
560,34	3	25827	111,1	1	26199	111	1	26199	111	1	36324	111
568,78	5	24885	108,1	4	25452	108	4	25452	108	3	35706	109,3
539,76	5	24735	107,6	4	25071	106,7	4	25071	106,7	3	34976	107,3
552,8	5	24543	107,1	7	24909	106,1	7	24909	106,1	1	34717	106,6
523,77	4	23718	104,8	2	24270	103,9	2	24270	103,9	4	33894	104,3
458,78	3	23168	103,2	5	24054	103,2	5	24054	103,2	7	33514	103,3
1075,51	1	23146	103,1	1	23860	102,6	1	23860	102,6	1	33493	103,2
552,3	2	22885	102,4	1	23610	101,8	1	23610	101,8	5	33285	102,6
857,9	5	22219	100,4	1	22894	99,5	1	22894	99,5	2	32427	100,3
436,77	3	22609	101,5	3	23393	101,1	3	23393	101,1	5	32716	101,1
1161,04	2	22136	100,2	2	22990	99,8	2	22990	99,8	1	32406	100,2
552,3	3	21729	99	3	22766	99,1	3	22766	99,1	2	32170	99,6
901,42	6	21785	99,2	2	22492	98,3	2	22492	98,3	2	31962	99
406,24	1	21649	98,8	1	22658	98,8	1	22658	98,8	1	31878	98,8
423,27	1	21628	98,7	1	22644	98,8	1	22644	98,8	3	31879	98,8
559,78	8	21246	97,6	9	22168	97,2	9	22168	97,2	1	31321	97,3
384,23	2	20774	96,3	3	21854	96,3	3	21854	96,3	1	30976	96,3
377,72	5	20753	96,2	1	21832	96,2	1	21832	96,2	1	30937	96,2
547,3	5	20864	96,5	3	21732	95,9	3	21732	95,9	2	31040	96,5
362,7	1	20688	96	1	21765	96	1	21765	96	1	30875	96,1
379,24	1	19857	93,6	1	21019	93,6	1	21019	93,6	2	29991	93,7
362,22	2	19865	93,6	3	21018	93,6	3	21018	93,6	1	29990	93,7
559,28	2	20179	94,5	5	21077	93,8	5	21077	93,8	3	29782	93,1
746,35	6	18949	90,9	2	20671	92,4	2	20671	92,4	4	28943	90,8
628,86	1	639	4,6	2	788	5,1	2	788	5,1	1	684	4,6
764,83	1	671	4,8	2	748	4,9	2	748	4,9	1	711	4,7

	MIX											
	I			II			III			IV		
m/z	76. [Count]	76. [Scan]	76. [Rt]	77. [Count]	77. [Scan]	77. [Rt]	93. [Count]	93. [Scan]	93. [Rt]	94. [Count]	94. [Scan]	94. [Rt]
350,98	2	24449	127,3	2	25060	126,6	2	22755	126,7	2	22613	127
350,98	4	24141	124,8	3	24813	124,7	4	22614	125,6	4	22343	124,9
810,99	3	23438	121,2	1	24105	121,1	2	21842	121,2	1	21740	121,3
767,46	4	23362	120,9	3	24138	121,2	3	21783	121	2	21678	121,1
780,47	3	23385	121	3	24200	121,4	3	21773	121	2	21639	120,9
855,51	3	23349	120,9	4	24205	121,4	3	21693	120,7	3	21564	120,7
736,44	1	23405	121	3	24053	120,9	1	21705	120,7	2	21559	120,6
723,43	1	23613	121,8	2	24262	121,6	2	21849	121,2	2	21545	120,6
789,48	2	23257	120,5	4	24142	121,2	1	22045	121,9	2	21527	120,5
744,96	1	23555	121,6	3	24139	121,2	2	21661	120,5	2	21518	120,5
885,43	1	22422	115,7	1	23085	115,9	3	20507	114,4	1	20600	115,6
736,83	4	21900	113,3	3	22541	113,4	3	20299	113,4	2	20130	113,1
560,34	2	21169	109,8	2	21859	110,5	2	19708	110,4	2	19545	110,1
568,78	2	21172	109,9	1	21399	108,4	2	19296	108,4	2	19462	109,7
539,76	1	20266	105,9	2	20845	106,1	2	18800	106,1	1	18761	106,2
552,8	1	20122	105,2	2	20812	106	2	18665	105,5	1	18526	105,1
523,77	1	19820	103,8	2	20367	103,9	3	18274	103,7	1	18299	103,9
458,78	1	19676	103,3	1	20440	104,2	2	18143	103,1	1	18231	103,6
1075,51	1	19434	102,2	1	19993	102,4	1	17977	102,3	1	17913	102,1
552,3	1	19311	101,6	1	19888	101,9	2	17861	101,8	4	17895	101,9
857,9	4	18837	99,6	5	19465	100,1	4	17494	100,1	3	17726	101,1
436,77	1	19019	100,3	2	19555	100,4	1	17559	100,4	2	17537	100,1
1161,04	1	18854	99,7	3	19382	99,9	1	17394	99,7	1	17400	99,5
552,3	1	18791	99,4	1	19312	99,6	2	17362	99,6	4	17366	99,4
901,42	3	18583	98,4	3	19116	98,7	4	17243	99	4	17168	98,4
406,24	1	18587	98,4	1	19093	98,6	1	17100	98,5	1	17141	98,3
423,27	1	18574	98,4	2	19064	98,5	1	17087	98,4	1	17122	98,2
559,78	2	18592	98,5	2	18876	97,6	3	16887	97,5	2	16918	97,2
384,23	2	18147	96,6	1	18579	96,2	1	16628	96,2	1	16680	96
377,72	1	18031	96	1	18562	96,2	1	16598	96	1	16650	95,8
547,3	3	18015	95,9	4	18433	95,7	3	16513	95,7	2	16604	95,6
362,7	1	17968	95,7	1	18465	95,8	2	16542	95,8	2	16590	95,6
379,24	1	17475	93,7	2	17970	93,9	1	16121	93,7	1	16200	93,7
362,22	2	17453	93,6	1	17919	93,7	2	16116	93,7	1	16155	93,5
559,28	1	17250	92,6	2	18141	94,6	1	16169	93,9	3	16034	92,8
746,35	2	16896	91	4	17204	90,7	1	15895	92,6	1	15727	91,3
628,86	3	718	5,4	2	777	5,4	2	802	5,5	2	763	5,7
764,83	2	765	5,7	1	828	5,6	1	708	5,2	1	708	5,4

Zusammenfassung

Der Feldhamster gehört zu den vorratssammelnden Winterschläfern und zeigt große individuelle Unterschiede im Winterschlafverhalten, welche vermutlich mit der Qualität der Vorräte zusammenhängen. Ziel dieser Studie ist es eine neue, nicht-invasive Methode zur Untersuchung der Ernährungsweise von Feldhamstern zu validieren. Dafür wurden massenspektroskopische Analysen spezifischer Peptidmuster von im Kot ausgeschiedenen Pflanzenteilen herangezogen. Hamster wurden unter kontrollierten Bedingungen mit bestimmten Mengen ausgewählter Futtersorten gefüttert. Kotproben wurden gesammelt, aus denen mittels Urea Proteine extrahiert und mit dem Massenspektrometer analysiert wurden. Mit Hilfe des Programm ProtMAX, hat ein Algorithmus die Spektralzählungen aller Peptidmerkmale mit dem gleichen Masse-Ladungs-Verhältnis (*m/z*) bei einer bestimmten Retentionszeit zusammengefasst. Dadurch können Peptidmerkmale, selbst ohne vorherige Identifikation, weiter analysiert werden. Dieser daten-unabhängige Ansatz heißt Mass Accuracy Precursor Alignment (MAPA). Nach anschließendem Filtern nach bestimmten Peptidmerkmalen, entsteht ein eigener peptide-mass-fingerprint (PMF) für jede Futtersorte. Ebenfalls wurden auch Peptidmerkmale, die in allen Proben vorkamen, als potentiell hamsterspezifische PMFs zusammengefasst, die dann eventuell zur Arterkennung verwendet werden können. Mit diesen PMFs haben wir pflanzenspezifische Biomarker kreiert, die zur Identifikation verdauter Pflanzenarten verwendet werden können und es ermöglichen dadurch die konsumierte Nahrung zu rekonstruieren. Mit Hilfe dieser Methode kann zwischen gelagerten und konsumierten Nahrungsbestandteilen unterschieden werden, wodurch individuelle Nahrungs- und Überwinterungsstrategien verglichen werden können. Die Verwendung dieser Technik in der Analyse von Hamsterkot, bei wildlebenden Hamstern, könnte die Kenntnisse über das Ernährungsverhalten der Feldhamster, insbesondere deren Umgang mit Nahrungsvorräten während des Winters, erweitern, was den Erfolg von Management- und Wiederansiedlungsprojekten entscheidend verbessern könnte.