Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

Lydia Staufenbiel[1]*, Anja-Elvira Müller[2] and Heidrun Gehlen[1]

[1]Horse Clinic, Freie Universität Berlin, Germany

[2]Anne-Frank-Straße 7/2, 71701 Schwieberdingen, Germany

*Corresponding Author: Lydia Staufenbiel, Horse Clinic, Freie Universität Berlin, Germany.

Abstract

In order to use faecal samples as means of laboratory diagnostics to analyze the mineral supply of organisms, it is necessary to define standardized sampling requirements. The present investigations monitor the variation of concentrations of mineral compounds within faeces as well as influences, such as age and storage of the samplings.

The faecal sample was separated horse dung of a female German Warmblood horse, which was appropriately provided with minerals according to supply recommendations. It was examined within nine different localizations, immediately after defecation, as well as 24 and 72 hours later. Moreover, six additional samplings of other horses were portioned into four different containers and stored for seven days at -18°C, -8°C, 8°C and 20°C. Afterwards, the samplings were dried and examined. With the help of ICP-OES and ICP-MS and due to standardized instructions 28 elements were identified. Furthermore, the relative error of the method was calculated.

Within the faeces, the concentration of identified elements is increasing from inner to outer regions. Considering the horizontal and vertical variation, the former showed significant differences between three, the latter one between eleven elements. The laps of time between defecation and sample taking significantly affected the concentration of mineral compounds in 16 cases, although the samplings do not vary until 24 hours after defecation. In contrast to that, there was an increase in the concentration of mineral compounds 72 hours after sample taking. The storage does not significantly influence the concentration of mineral compounds of the faeces, except from two elements.

The increase in mineral compounds together with rising sampling age and further distance to the centre of the heap is due to the relative rise, after volatile compounds decreased. The storage does not have an effect on the traceable concentration and thus, the two divergent elements are attributed to random variation. Moreover, the existing stability of concentrations in faecal samples does have a clinical relevance and, accordingly, a standardised procedure should consider sample taking of more central localizations of currently excreted dung. Additionally, in case of long-lasting storage, the samples should be kept chilled. The only exception to diverge from this procedure is the analysis of samples taken from the center of the dung within three days after defecation.

Keywords: Storage; Age of Samples; Variation; Analysis of Mineral Compounds; Horse; Faeces

Abbreviations

GfE: Gesellschaft für Ernährungsphysiologie/Society of Nutrition Physiology; ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; n.s.: Not Significant

---

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

33

Introduction

After the publication of the demand recommendations of the Society of Nutrition Physiology 2014 [1], some rations in German horse stables may have been recalculated. However, this requires an analysis of the ingredients of the basic feedstuffs, especially with regard to the mineral supply, as these are not declared in nature and the content of quantity and trace elements varies greatly. This is cost-intensive and must be arranged by the farm manager for horses kept in boarding stables. So the horse owners have only limited possibilities to calculate the ration adequately. Furthermore, feed intake of horses depends on many animal and environmental factors, making it difficult to track accurate intake for these selective eaters anyway [2].

In dairy cows, the analysis of faecal samples has been recommended as an extension of the range of methods to monitor supply of quantity [3] and trace elements [4], with reference to elemental differentiation of diagnostic significance. There is a current case study of chronic copper oversupply in a dairy herd of Weber *et al.* from 2021. The authors compare the copper concentrations in feed rations (Total mixed ration) in whole blood, plasma, serum, urine, hair, liver, and faecal samples and conclude that TMR as well as liver and faecal analyses indicate copper oversupply with a clear value excursion in contrast to the other sample materials [5]. These results for cattle raise the question of whether faecal analyses can also be used in horses as an extension of the diagnostic method tool for monitoring the supply of quantity and trace elements. Since there are no systematic studies on this method so far, the first step is to clarify the methodological boundary conditions for the collection and treatment of the dung samples up to the laboratory analysis.

The location of the faecal sample collection (from the cranial rectum during a rectal examination, ampulla recti, and freshly deposited dung pile) has no significant influence on the measured faecal concentrations of quantity and trace elements. Therefore, with regard to a simple practical application, the faecal sample collection from the dung pile is recommended [6]. From this recommendation result several further questions concerning the methodological implementation, which will be investigated in this publication: 1. location-related distribution of the concentrations of the quantitative and trace elements in the dung pile (nine locations); 2. influence of the storage period (0, 1, 3 days) of the dung pile outdoors on the element concentrations; 3. influence of the storage temperature (room temperature +20°C, refrigerator +8°C, freezer -8°C and -18°C) after sampling on the analyzed element concentrations. In addition, repeat measurements of the same faecal sample were used to calculate the reproducibility of the measured values for the entire process from sample collection to final analysis.

Materials and Methods

The sample material was a freshly settled pile of faeces from a 15-year-old German sport horse mare. This has been located on a sand paddock and has been protected by a fence from manipulation by other horses. The subject has been adequately supplied with minerals according to the recommendations of the GfE [1]. Sampling from this pile has been performed at three different times in different layers and locations. Samples have been taken on day 0, immediately after weaning, day 1 (24 hours) and day 3 (72 hours later). The weather at this time has been mild to hot with single showers. For the sampling sites has been first the stratification, i.e. the vertical component distinguished into top, middle and bottom. Furthermore, a classification of the horizontal plane from the center to the outside has been made (Figure 1).

Furthermore, the sampling has been performed of six different horses, that have been randomly divided into three feeding groups [6]. From each horse, the sample has been divided into four vessels, which have subsequently been stored for seven days at room temperature (20°C), refrigerated (8°C), frozen (-8°C) and deep frozen (-18°C) before drying in a warming cabinet for 48 hours at +60°C. Samples ready for storage by drying were transferred to the IDEXX laboratory in Ludwigsburg, where the measurement of 28 elements was performed according to certified standard instructions using ICP-OES (Cu, Zn, Mn, Mo, Ca, Mg, Ba, Cr, Fe, Sr, K, S, Ti, P, Si, and Na) and ICP-MS (Se, B, Co, Ni, Pb, Cd, U, As, Tl, Al, Sn, and Li), respectively. More details on the methodology can be found in [7] and further literature.
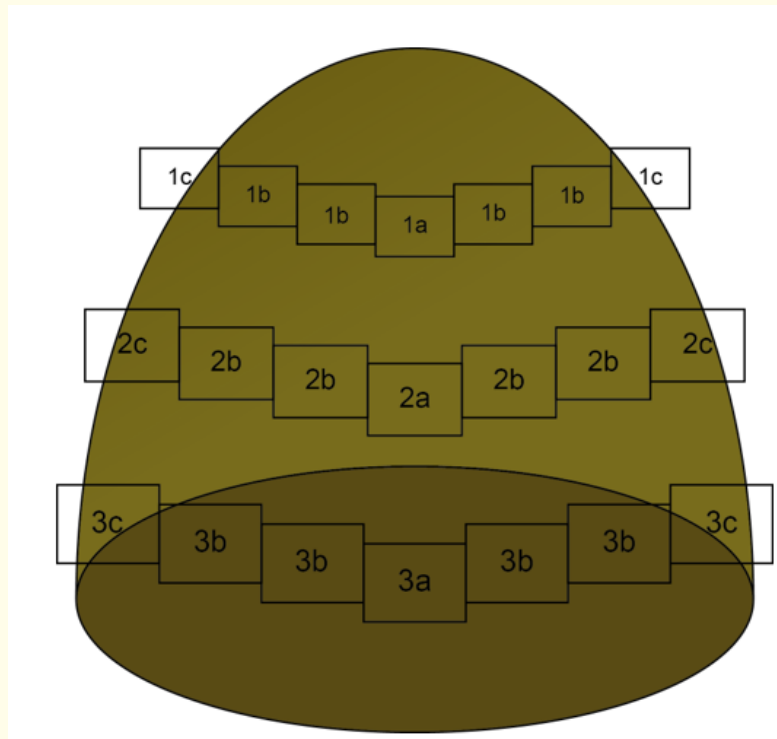
Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

34

Figure 1: *Draft of a heap for clarification of the sample collecting, to investigate the influence on different concentrations of mineral compounds in different localization. The vertical locations are numbered, the horizontal ones labelled with letters (two time "b", because of collecting to samples out of the middle).*

Statistical analysis

The data collection and management was performed using Microsoft Excel 2016 (Microsoft Corporation), and the statistical analysis was performed using SPSS Statistics 25 (IBM), in which the figures were also generated.

A total of 36 faecal samples were collected from a dung pile. Three fixed factors can be distinguished as grouping variables: horizontal stratification (three expressions), vertical stratification (three expressions), and sample day (time between excretion and sample collection in three expressions). The distribution of sampling locations is outlined in figure 1. The effect of these three fixed factors was performed in one computational run using the linear mixed models, analysis of variance with fixed effects [8]. Due to the size, the statistical analysis results were listed separately in the three table 1-3. Four model variants were successively calculated for the 28 different elements, once with only the three fixed factors horizontal stratification, vertical stratification, sample day without interaction and once with the three factors and optionally including the interaction day/horizontal stratum or day/vertical stratum or both interactions. By subsequently testing the normal distribution of the residuals with the Shapiro-Wilk test (Table 3), the model with the best approximation to the normal distribution was selected. In addition, to assess the homoscedasticity, the residuals were plotted graphically against the values predicted by the model and their distribution was assessed visually. The level of significance were classified in: < 0.001 (highly significant), < 0.01 (very significant) and < 0.05 (significant). In this case, a post hoc LSD test (t-test) ($p < 0.05$) followed for pair wise

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

35

comparison of mean values; results were marked with lowercase letters as index. Significantly different means are marked by different superscript letters, identical letters represent the absence of a significant difference.

To evaluate the influence of sample storage (Table 4), a univariate analysis of variance (general linear model) with repeated measures was performed. The factor storage entered the model as a measurement repetition with four expressions (-18°C, -8°C, 8°C, room temperature). If significance was demonstrated ($p < 0.05$), a pairwise comparison of the four mean values was performed using Bonferroni's post hoc test ($p < 0.05$), and the results were marked with lowercase letters as an index. Significantly different means are marked by different superscript letters, and equal letters represent the absence of a significant difference. The influence of the six horses is reported as a between-subjects effect in the model and listed as supplementary information in table 4. Horses from different feeding groups were selected to increase the between-subject variance (Figure 2 and 3).

The relative error (Table 5) was calculated from the 36 individual measurements from table 3. Due to the significant effect of sample day, the mean values were calculated for the three sample days (0, 1, 3 days after defecation) from the twelve individual readings per day. Then, the difference of individual reading and mean value was calculated for the three days. The quotient of the absolute value of this difference and the mean value gives the relative error (relative error = [(|measured value A - mean value on sample day 0/1/3|)/(0.5 measured value A + 0.5 measured value on sample day 0/1/3)]. From the 36 numerical values for the relative error for each of the 28 elements, the mean, median, and 25% and 75% quantile values were calculated (Table 5). The relative error includes all sources of variation in the overall process from sampling at different locations in the dung pile, through storage, processing including drying, and chemical analysis.

Results

Table 1 shows the influence of horizontal dispersion within the faecal pile from the inside to the outside. It should be noted that two samples were taken from different locations of the middle between the center and the outer zone. These have been combined, resulting in the center/middle/outside distinction for the localizations (Figure 1). Significant differences have been found for magnesium, copper and aluminum, with "outside" being the significantly higher concentration measured (marked with letter "b"). Looking only at the numerical values, faecal concentrations for all six quantity elements in the "outside" region are higher or equal to the other two sampling locations. This observation result is also true for 17 of the 22 trace elements analyzed (Table 1).

	Mean value 1	Mean value 2	Mean value 3	Standard error 1+3	Standard error 2	Level of significance
n	9	18	9			
Quantity elements in g/kg dry matter						
Ca	7,1	7,6	8,5	0,49	0,35	n.s.
P	4,6	5,0	5,4	0,23	0,17	n.s.
Mg	4,2[a]	4,1[a]	4,7[b]	0,17	0,12	<0,05
Na	5,1	5,0	5,1	0,16	0,12	n.s.
K	5,2	5,1	5,4	0,16	0,12	n.s.
S	1,9	1,9	1,9	0,03	0,02	n.s.
Trace elements in mg/kg dry matter						
Fe	575	583	610	12,7	9,0	n.s.
Cu	13,8[a]	14,0[a]	15,3[b]	0,40	0,28	<0,05
Zn	100	100	108	4,7	3,4	n.s.

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

36

Mn	124	137	149	10,8	7,6	n.s.
Se	0,05	0,07	0,06	0,056	0,039	n.s.
Co	0,30	0,30	0,32	0,008	0,005	n.s.
Cr	0,59	0,69	0,66	0,044	0,031	n.s.
Mo	2,44	2,44	2,55	0,084	0,059	n.s.
Si	2857	2593	2668	134,3	94,9	n.s.
B	19,6	19,2	29,6	0,50	0,36	n.s.
As	0,23	0,23	0,25	0,009	0,006	n.s.
Ni	1,10	1,11	1,18	0,038	0,027	n.s.
Sn	0,15	0,15	0,14	0,021	0,015	n.s.
Ba	47,4	49,1	50,0	1,19	0,84	n.s.
Sr	38,6	43,0	43,3	2,35	1,66	n.s.
Pb	1,99	1,98	2,00	0,070	0,049	n.s.
Cd	0,17	0,17	0,17	0,008	0,006	n.s.
U	0,09	0,09	0,09	0,006	0,004	n.s.
Tl	0,55	0,56	0,59	0,015	0,011	n.s.
Al	406[b]	354[a]	402[b]	15,3	10,8	<0,05
Ti	23,0	22,0	21,8	0,84	0,59	n.s.
Li	1,07	1,00	1,08	0,031	0,022	n.s.

Table 1: *Influence of horizontal variation on samplings (from central to outside); 1= Central (n = 9),
2= Middle (n = 18, because of combining two sampling locations), 3= Outside; significant differences in the
paired comparison are tagged with indices; n = 36.*

Analogous to the horizontal dispersion, the vertical dispersion from top to bottom has also been examined (Table 2). Here, for a total of 11 of the 28 determined elements (4 of 6 quantity elements, 7 of 17 trace elements) a significant influence of the layer is present. Whereby in all cases the significantly lowest concentration (marking with letter "a") is found in the localization "middle". With the only exception of aluminum, the significantly highest concentrations of faecal elements (marked with letter "b") are found in the lower layer, being equal to those in the upper layer in eight cases (Table 2).

	Mean value 1	Mean value 2	Mean value 3	Standard error	Level of significance
n	12	12	12		
Quantity elements in g/kg dry matter					
Ca	7,2	8,1	7,8	0,43	n.s.
P	5,2	4,8	5,0	0,21	n.s.
Mg	4,4[b]	4,0[a]	4,7[b]	0,15	<0,05
Na	4,8[a]	5,1[ab]	5,5[b]	0,15	<0,01
K	5,3[b]	4,8[a]	5,6[b]	0,15	<0,01
S	2,0[b]	1,8[a]	2,0[b]	0,03	<0,001

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

37

Trace elements in mg/kg dry matter				
Fe	609[b]	560[a]	599[b]	11,2	<0,01
Cu	14,2[a]	13,4[a]	15,6[b]	0,35	<0,01
Zn	100	101	107	4,2	n.s.
Mn	143	132	134	9,5	n.s.
Se	0,02	0,07	0,09	0,049	n.s.
Co	0,32	0,30	0,30	0,007	n.s.
Cr	0,66	0,63	0,65	0,039	n.s.
Mo	2,55	2,37	2,51	0,074	n.s.
Si	2659[a]	2459[a]	3001[b]	118,4	<0,05
B	20,1	18,6	19,8	0,44	n.s.
As	0,24	0,23	0,23	0,008	n.s.
Ni	1,16	1,13	1,10	0,034	n.s.
Sn	0,17	0,14	0,12	0,018	n.s.
Ba	51,5[b]	45,0[a]	49,8[b]	1,05	<0,01
Sr	41,3	43,1	40,4	2,07	n.s.
Pb	2,07	1,89	1,94	0,061	n.s.
Cd	0,19[b]	0,15[a]	0,17[b]	0,007	<0,01
U	0,09	0,09	0,09	0,005	n.s.
Tl	0,60[b]	0,52[a]	0,59[b]	0,014	<0,05
Al	431[b]	362[a]	369[a]	13,5	<0,01
Ti	21,9	21,3	23,6	0,74	n.s.
Li	1,09	0,99	1,06	0,027	n.s.

Table 2: *Influence of collecting layers from top to bottom; 1= Top, 2= Middle; 3= Bottom; significant differences in the paired comparison are tagged with indices; n= 36.*

To investigate the influence of the elapsed time between excretion and sampling, samples were collected directly from the freshly settled pile, 24 and 72 hours later. Here, a significant influence is found for a total of 16 of the 28 elements determined, with the quantity elements standing out again, which were all significantly influenced by sample age, with the exception of calcium (Table 3). The values of the significant differences between the sampling times show a clear trend. The measured values of the samples taken from the freshly settled faecal pile (time 0h) are significantly lowest (or equal) for all 16 elements compared to the later sampling times (marked with letter "a"). Conversely, significantly higher concentrations of faecal elements (marked with letter "b" or "c") compared to sampling from the fresh faecal pile are found in all 16 cases in the dung samples collected after 72 h of outdoor storage (Table 3).

	Mean value 0h	Mean value 24h	Mean value 72h	Standard error	Level of significance	Shapiro-Wilktest residues
n	12	12	12			
Quantity elements in g/kg dry matter						
Ca	8,0	7,1	8,0	0,43	n.s.	0,606
P	4,5[a]	4,6[a]	5,6[b]	0,21	<0,01	0,590
Mg	4,2[a]	4,2[a]	4,8[b]	0,15	<0,01	0,267
Na	4,8[a]	5,2[b]	5,3[b]	0,15	<0,05	0,353
K	5,0[a]	5,1[a]	5,7[b]	0,15	<0,01	0,046
S	1,9[a]	1,8[a]	2,1[b]	0,03	<0,001	0,284

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

38

Trace elements in mg/kg dry matter					
Fe	554[a]	568[a]	646[b]	11,6	<0,001	0,652
Cu	12,9[a]	14,1[b]	16,1[c]	0,37	<0,001	0,748
Zn	94[a]	99[ab]	115[b]	4,35	<0,01	0,266
Mn	148	119	143	9,8	n.s.	0,295
Se	0,03	0,01	0,14	0,051	n.s.	0,001
Co	0,31	0,29	0,31	0,007	n.s.	0,894
Cr	0,61	0,60	0,73	0,040	n.s.	0,767
Mo	2,30[a]	2,39[a]	2,74[b]	0,077	<0,01	0,907
Si	2456[a]	2597[a]	3066[b]	122,6	<0,01	0,870
B	19,4	18,8	20,3	0,46	n.s.	0,676
As	0,24	0,24	0,23	0,008	n.s.	0,505
Ni	1,21	1,10	1,07	0,035	n.s.	0,522
Sn	0,17	0,13	0,13	0,019	n.s.	0,137
Ba	44,9[a]	45,8[a]	55,7[b]	1,09	<0,001	0,301
Sr	42,8	38,4	43,6	2,14	n.s.	0,070
Pb	1,84[a]	1,94[a]	2,13[b]	0,063	<0,05	0,163
Cd	0,16[a]	0,16[a]	0,19[b]	0,007	<0,05	0,039
U	0,09	0,08	0,093	0,006	n.s.	0,257
Tl	0,52[a]	0,55[a]	0,64[b]	0,014	<0,001	0,067
Al	362[a]	342[a]	458[b]	14,0	<0,001	0,620
Ti	21,5	21,5	23,8	0,76	n.s.	0,363
Li	1,00[a]	0,99[a]	1,16[b]	0,003	<0,001	0,703

Table 3: *Influence of time between defecation and collecting the samples, significant differences are tagged with indices; n = 36.*

After sampling, the faecal samples must be temporarily stored until processing by drying in preparation for laboratory analysis. Figure 2 and 3 show examples of the influence of differentiated storage temperatures for a storage period of seven calendar days on the faecal concentrations of calcium and copper. The measured values vary unordered between the storage variants. Statistical testing shows no significant effect of tested storage conditions on measured faecal concentrations for 26 of the 28 elements analyzed (Table 4). Mean value 1, immediate freeze storage at -18°C after sample collection, can be used to compare the effect of higher sample storage temperature. A significant effect of storage temperature is shown for the quantity elements potassium and sulfur, although no consistent trend in the distribution of values between storage temperatures can be seen (Table 4).

	Mean value 1	Mean value 2	Mean value 3	Mean value 4	Level of significance storgae	Level of significance horses
n	6	6	6	6		
Quantity elements in g/kg dry matter						
Ca	7,2	6,8	7,0	7,6	n.s.	0,005
P	5,9	4,5	4,9	5,2	n.s.	0,000
Mg	4,2	4,0	4,4	4,4	n.s.	0,000
Na	3,5	2,9	3,8	3,6	0,111	0,007
K	6,3[ab]	5,7[a]	6,8[b]	6,7[ab]	<0,05	0,000
S	2,6[ab]	2,2[a]	2,6[b]	2,7[b]	<0,05	0,000

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

39

Trace elements in mg/kg dry matter						
Fe	1535	1701	1558	1547	n.s.	<0,05
Cu	17,3	15,6	17,0	17,1	n.s.	<0,01
Zn	104	92	92	107	n.s.	<0,001
Mn	258	242	234	265	n.s.	<0,05
Se	0,10	0,12	0,11	0,14	n.s.	<0,05
Co	0,55	0,58	0,54	0,64	n.s.	n.s.
Cr	1,93	2,75	2,40	2,02	n.s.	n.s.
Mo	2,01	1,72	1,58	1,56	n.s.	<0,001
Si	4375	10625	8797	5084	n.s.	<0,001
B	16,4	18,6	16,6	18,8	n.s.	<0,05
As	0,53	0,52	0,48	0,58	n.s.	<0,05
Ni	1,68	1,77	1,65	1,98	n.s.	n.s.
Sn	0,08	0,28	0,12	0,40	n.s.	n.s.
Ba	67,6	67,8	68,2	71,8	n.s.	<0,01
Sr	57,9	49,6	50,3	57,3	n.s.	<0,05
Pb	3,53	3,58	3,18	3,29	n.s.	n.s.
Cd	0,39	0,32	0,30	0,30	n.s.	0,05
U	0,07	0,12	0,08	0,101	n.s.	n.s.
Tl	0,28	0,27	0,23	0,25	n.s.	<0,05
Al	519	1096	757	1141	n.s.	n.s.
Ti	33,1	94,8	65,4	46,9	n.s.	n.s.
Li	0,99	1,59	1,19	1,61	n.s.	<0,05

Table 4: *Arithmetic means of differently stored samples: 1= -18°C, 2= -8°C, 3= 8°C, 4= 20°C. The statistical significance for horses is listed separately because of different feedings groups and the temperature, the samples were stored at; n = 24.*
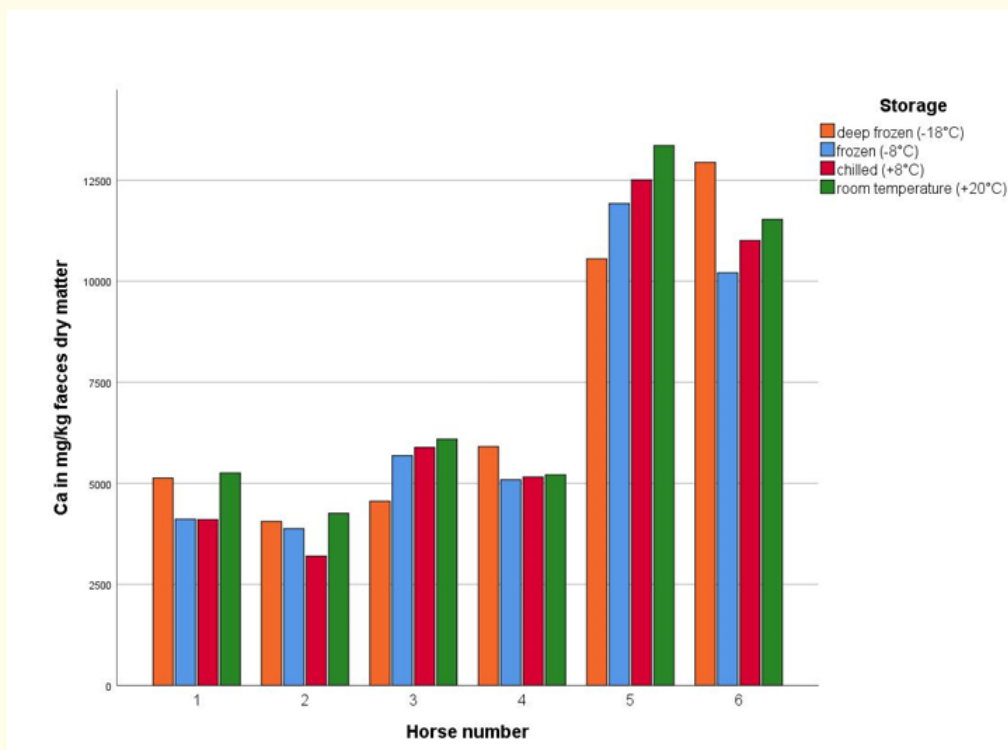

Figure 2: *Influence of storage on the concentration of calcium in samples; Horses number 1 and 2 out of feeding group 1 (selected concentration of minerals); horses 3 and 4 feeding group 2: no mineral supplementation; horses 5 and 6 get minerals for free intake.*
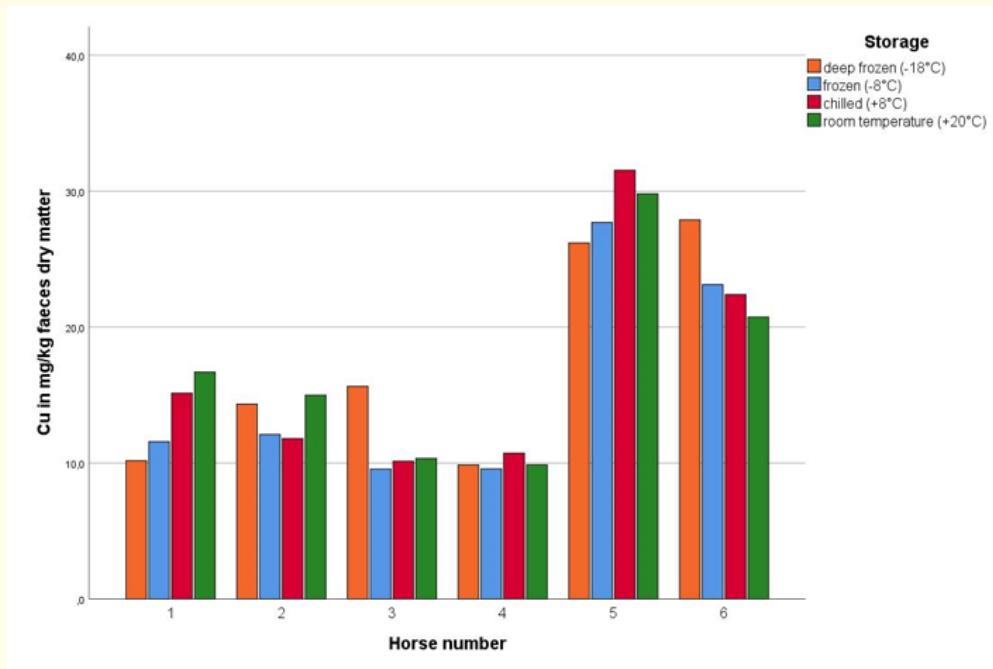
Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

40

Figure 3: *Copper like figure 2.*

In the dung pile (Figure 1), the concentrations of the six quantity and 22 trace elements were measured 36 times repeatedly. The relative error was calculated from the differences between the measured values of the 36 individual values and the mean value (Table 5). As a relative number, it indicates the expected deviation of a measured value from the mean value. The variation in the overall procedure from faecal sample collection to storage, sample drying and laboratory analysis is included in the calculation. With the exception of selenium (0.60) and tin (0.27), the median values are below 0.20 for the other 26 elements.

	Mean value	Median	25%-Quantile	75%-Quantile
Quantity elements				
Ca	0,163	0,140	0,057	0,280
P	0,122	0,110	0,041	0,181
Mg	0,117	0,105	0,051	0,165
Na	0,112	0,082	0,026	0,134
K	0,101	0,067	0,027	0,153
S	0,066	0,056	0,026	0,100
Trace elements				
Fe	0,070	0,052	0,016	0,089
Cu	0,098	0,080	0,034	0,138
Zn	0,134	0,127	0,057	0,180
Mn	0,177	0,142	0,051	0,242

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

41

Se	0,726	0,600	0,211	0,917
Co	0,067	0,037	0,018	0,106
Cr	0,183	0,133	0,052	0,243
Mo	0,089	0,085	0,029	0,128
Si	0,163	0,154	0,076	0,235
B	0,060	0,042	0,014	0,074
As	0,084	0,074	0,024	0,111
Ni	0,083	0,076	0,038	0,118
Sn	0,360	0,270	0,098	0,486
Ba	0,094	0,076	0,046	0,119
Sr	0,118	0,077	0,042	0,168
Pb	0,097	0,084	0,037	0,138
Cd	0,158	0,131	0,076	0,201
U	0,145	0,132	0,047	0,217
Tl	0,101	0,081	0,065	0,124
Al	0,139	0,131	0,046	0,196
Ti	0,109	0,074	0,031	0,156
Li	0,080	0,058	0,031	0,128

Table 5: *Relative faulta of the investigation of element concentrations from the whole process beginning with recovery of samples, reconditioning and analysis (n = 36).*
arelative fault = [(|value A – value B|) / (0,5 value A + 0,5 value B)].

Discussion

The dry matter content in the faeces varies in a wide range between 167 to 335 g/kg [2], which is why the reference to the dry matter is of great relevance for the evaluation of the comparability of the measured values. Although faecal analyses are used, especially in animal nutrition, to question the digestibility of minerals little information is found in the literature on the marginal conditions of sample collection. However, for the use of faecal samples for the analysis of mineral supply, these must be clarified and standardized so that reference values can be determined in the future.

The first issue of central importance for faecal sampling relates to possible both random and ordered differences in concentration of individual quantity and trace elements within the dung pile. Ideally, sampling anywhere in the faecal pile would give the same measurement result.

Horizontal dispersion in the dung pile had a significant effect on only three of the 28 elements measured, magnesium, copper and aluminum. For all three elements, the concentration from the samples collected on the outside has been the highest (Table 1). The magnesium compounds (oxide and carbonate) [9], as well as copper II suflate pentahydrate, contained in the supplemental feeds are poorly soluble in water [10], so it can be assumed that the compounds contained in the faeces were not dissolved by the rain, but rather were much more likely flushed to the outside. This could explain the analogous distribution of copper and magnesium in the faecal pile. With regard to aluminum, it is more of a random sample effect, as the concentrations are the same on the outside and in the center (Table 1).

For all element concentrations significantly differing within the stratum (Mg, Na, K, S, Fe, Cu, Si, Ba, Cd, Tl, Al), statistically the lowest concentration was found in the central stratum without exception (Table 2). This portion is the least exposed to weathering and soil con-

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

42

tact, so there is no decline in the soluble constituents as described below, and thus no relative increase in mineral content.

The second question of central importance to faecal sampling is directed to the length of time from weaning of the faecal pile to sample collection. It is an important finding that significant changes in faecal concentrations occur in a three-day period for five of the six quantity elements and eleven of the 22 trace elements studied (Table 3). It is noteworthy that these concentration changes are not irregular, but follow a consistent pattern for all elements. Looking at both statistical and numerical values, the highest concentrations are found in the oldest faecal samples. Those that have been outdoors for three days (Table 3). In contrast, with the exception of sodium and copper, the faecal concentrations of the measured quantity and trace elements are statistically the same on day 0 and day 1, i.e. within 24 hours of excretion. This is a very important finding for the subsequent possibility of routine analysis.

The predominant trend of increasing concentration with increasing age of the samples is probably best explained by the relative shift of the contents in the dry matter. Dry matter in horse manure averages 240 g/kg [2], 890 g/kg of which is on average accounted for by organic matter and just 110g/kg by crude ash, which contains the minerals (proximate analysis, [11]). Among others the contents of the organic matter represent, the carbohydrates, which are very water soluble due to the many hydroxyl groups [12]. Since, as described, there was light precipitation during the three days the samples were lying, one can assume a volatilization of this fraction, accounts for an average of 380 g/kg of the dry matter in the faeces, according to [2]. Since element concentrations in the faecal samples have been measured as a proportion of the dry matter, a relative increase in elements can thus be explained by a decrease in organic matter in the dry matter.

This explanatory approach can be extended to the results in table 1-3 as a common central factor. The concentration pattern can be summarized as equal or lower concentrations of quantity and trace elements being measured in the center of the dung pile during short storage periods, and conversely equal or higher concentrations being measured in the outside (top, bottom, side) dung regions, increasing with longer storage periods. But it must be emphasized that regardless of statistical significance evidence; the absolute value expressions of concentration gradients are low, which allows a recommendation for reliable practical implementation of sample collection.

Regardless of storage, the samples are stable for seven days, which can also be seen in table 4, figure 2 and 3. In the case of potassium and sulfur, random variation can be assumed, as otherwise no trend is apparent. It should be emphasized, however, that the samples were stored in a closed container and, at the variant room temperature, in the shade. This aspect is practically relevant, since no refrigerated sample transport is necessary, but for hygienic reasons the refrigerated variant is certainly to be preferred for longer storage.

The relative errors reported in table 5 are small, with median values below 0.2. They underline the practicability of the test methodology as an overall procedure. By multiplying a measured value by the magnitude of the relative error, the distance to a mean value determined from repeat measurements of the same sample can be estimated. This estimated value has the special feature of including all methodological sources of error from the collection of the faecal sample, intermediate storage, drying and sub sampling from the dried sample to the laboratory analytical performance ICP-OES or ICP-MS measurement. Such comprehensive error data are the exception in clinical chemistry. The relative errors exceed 0.2 only for tin (0.27) and especially for selenium (0.60) (Table 5). Both elements are found in the faecal samples only at low concentrations below 1 mg/kg faecal TS (Table 3), with selenium being the element with the lowest concentration of all 28 elements analyzed. Some individual readings are below the detection limit for selenium and are therefore recorded as 0 mg/kg faecal TS. Thus, the selected method for calculating the relative error for selenium has limited applicability. However, it should not be concluded from this that selenium determination is not applicable for faecal testing. Above a lower limit value to be defined, selenium measurement values should be evaluated as undetectable and excluded from statistical evaluations. This specificity for selenium matches good with the metabolic characteristics of a high absorption rate and relevant renal excretion [13].

Investigation of the Variation of Concentration of Quantity and Trace Elements in Equine Faecal Samples Considering Storage

43

Conclusion

For faecal concentrations of various quantity and trace elements, there is a statistically significant trend toward an increase in measured values with faecal sampling in the outer region of the dung pile compared to the center, as well as with increasing distance from the time of faecal collection. However, from the point of view of a practical application, the numerical differences in values are small, indicating the stability of the dung samples. The aim should be to standardize the sampling. Therefore, the recommendation is to collect the dung samples from a freshly settled pile from the center. This will most accurately reflect the amount of quantity and trace elements contained, as these samples are not subject to varying weather conditions. In exceptional cases, it is permissible to obtain faecal samples up to three days old from the central dung pile. The stored faecal samples show a high stability even at room temperature over 7 days, nevertheless, a sample storage cooled or frozen is to be preferred also for hygienic reasons.

Bibliography

1. Gesellschaft für Ernährungsphysiologie. "Empfehlungen zur Energie- und Nährstoffversorgung von Pferden". Energie-und Nährstoffbedarf landwirtschaftlicher Nutztiere. Frankfurt am Main: DLG-Verl (2014).

2. Coenen M and Vervuert I. "Pferdefütterung". 6. Aufl. Stuttgart: Thieme (2019).

3. Herold A., *et al.* "Konzentrationen an Mengenelementen beim Rind in verschiedenen Probenmedien unter besonderer Berücksichtigung von Kotproben". *Tierarztl Prax Ausg G Grosstiere Nutztiere* 46.4 (2018): 221-228.

4. Herold A., *et al.* "Konzentration von Spurenelementen beim Rind in verschiedenen Probenmedien unter besonderer Berücksichtigung von Kotproben". *Tierarztl Prax Ausg G Grosstiere Nutztiere* 48.01 (2020): 5-14.

5. Weber J., *et al.* "Chronische Kupferüberversorgung als mögliches Bestandsproblem in einer deutschen Milchviehherde". *Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere* 49.03 (2021): 203-209.

6. Staufenbiel L., *et al.* "Methodische Untersuchung zu Mengen- und Spurenelementkonzentrationen in Pferdekotproben unter besonderer Berücksichtigung des Probenentnahmeortes". *Tierarztl Prax Ausg G Grosstiere Nutztiere* 49.3 (2021): 178-188.

7. Mueller A., *et al.* "Differences in the selenium supply of horses across Europe". *Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere* 40.3 (2012): 157-166.

8. Bühl A. "SPSS 23. Einführung in die moderne Datenanalyse. Bd. 4297. Always learning. 15. Aufl. Hallbergmoos: Pearson (2016).

9. Sicius H. "Erdalkalimetalle: Elemente der zweiten Hauptgruppe". In: Handbuch der chemischen Elemente. Springer Spektrum, Berlin, Heidelberg (2020): 1-63.

10. Sicius H. "Kupfergruppe: Elemente der ersten Nebengruppe. In: Sicius H, Hrsg. Handbuch der chemischen Elemente. Berlin, Heidelberg: Springer Berlin Heidelberg (2019): 1-40.

11. Dobenecker B and Kamphues J Hrsg. "Supplemente zur Tierernährung. Für Studium und Praxis". 12. Aufl. Hannover, Germany: M. and H. Schaper; Meyer, Helmut (2014).

12. Wachter H and Hausen A. "Chemie für Mediziner. Wachter, Arno Hausen. (de Gruyter-Lehrbuch). 4. Aufl. Berlin: De Gruyter (1982).

13. Vervuert I and Stoebe S. "Selen beim Pferd im Spannungsbogen zwischen marginaler und toxischer Versorgung". *Pferdespiegel* 16.01 (2013): 27-33.

Volume 6 Issue 10 October 2021