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BLOOD AND HAIR AS NON-INVASIVE TRACE ELEMENT BIOLOGICAL INDICATORS IN GROWING RABBITS

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Abstract: The suitability of blood and hair as non-invasive tools to monitor trace element contents was studied in 48 Hyla male growing rabbits. Three diets with increasing organic selenium (Se) addition (0.1, 0.5 and 2.5 mg/kg) were used to induce alterations in the concentrations of trace elements *vs*. an unsupplemented diet. In blood, a linear decrease in Co (P<0.001), Cu (P<0.001), Mn (P<0.05), Zn (P<0.05), Sb (P<0.001), As (P<0.001), Cr (P<0.001), Mo (P<0.001), Ni (P<0.001) and Cd (P<0.001) concentrations with increasing dietary Se was observed. In hair, a cubic effect of dietary Se on Co (P<0.01), Cu (P<0.05), Mn (P<0.001), Pb (P<0.05), Mo (P<0.05) and Cd (P<0.05) concentrations was found, while As, Cr and Ni concentrations decreased linearly (P<0.01, P<0.01 and P<0.001, respectively) with increasing dietary Se. Selenium was negatively correlated to Sb, As, Cr, Mo, Ni and Cd, (P<0.001) in blood, and to As (P<0.05), Cr, Ni (P<0.01) and Pb (P<0.05) in hair. The contents of Se, As, Cr and Ni in blood were highly correlated (P<0.001) to those in hair. Blood appeared to be more sensitive than hair in detecting small changes in the trace element profile in rabbits, as was indicated by the discriminant analysis. In conclusion, blood and hair can be suitable biological indicators of essential, toxic and potentially toxic trace element status in rabbits, particularly when used complementarily.

Key Words: biological indicators, blood; hair, organic selenium, rabbits, trace elements.

INTRODUCTION

Biological indicators of heavy metal exposure are a vital tool to biomonitor the degree of exposure and to implement strategies to protect animals, humans and the environment. Trace element determination in feed, food and animal tissues can demonstrate both the potential exposure to heavy metals and the uptake of essential elements via the food chain. Heavy metal concentration in animal tissues may reflect the severity of local environmental pollution (De Temmerman *et al.,* 2003), as indicated by several studies in terrestrial (Miranda *et al.,* 2005; Waegeneers *et al.,* 2009) and aquatic (Milošković and Simić, 2015) organisms. Heavy metals differ from other pollutants in that they are neither created nor destroyed (Keil *et al.,* 2011) and may naturally occur at relatively high concentrations in the environment (Waegeneers *et al.,* 2009). The chemical form in which toxic elements are present may alter during passage through the intestine or during storage in tissues, but they are not metabolised (Kan and Meijer, 2007). Thus, translocation through the food chain is a potential threat for both animals and humans (López-Alonso *et al,* 2002; McDowell, 2003; Żukowska and Biziuk, 2008).

In rabbits, research on essential and toxic trace element levels in different tissues is scarce (Čobanová *et al*., 2018). In a recent work, liver and muscle tissues were used to study toxic and potentially toxic elements following dietary Se administration in rabbits (Papadomichelakis *et al.,* 2018). The results indicated that the potential of Se to reduce the concentration of toxic and potentially toxic elements was evident in both tissues, but most notable in liver. To this

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end, such an investigation in blood and hair would be interesting in rabbits for two reasons. Firstly, the production cycle in meat rabbits is short; approximately 42 d post weaning. Secondly, blood and hair are obtained with non-invasive methods and collection can be repeatedly carried out at any time prior to the end of the rearing cycle. For this purpose, blood and hair samples were collected before the end of the rearing cycle from the same animals used in our previous study (Papadomichelakis *et al.,* 2018) and the concentration of cobalt (Co), manganese (Mn), antimony (Sb), molybdenum (Mo), arsenic (As), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), iron (Fe), zinc (Zn) and cadmium (Cd) was determined. The hypothesis that any alterations in the elemental profile due to dietary Se administration would be adequately reflected in both tissues was tested. In addition, the trace element interrelationships within and between blood and hair were assessed, so as to determine the suitability of blood and hair as trace element biological indicators.

MATERIALS AND METHODS

Table 1: Ingredient and chemical composition of the basal diet (g/kg as fed basis).

	Basal diet
Ingredient	
Dehydrated alfalfa	294.0
Barley grain	170.0
Wheat bran	284.0
Sunflower meal, 280 g CP/kg	154.0
Citrus pulp	80.0
L-Lysine HCl, 80%	2.6
DL-Methionine, 99%	2.3
L-Threonine	2.0
Sodium chloride	4.1
Ultrafed® (binder)[a]	3.5
Mineral-Vitamin premix[b]	3.5
Calculated chemical composition	
Dry matter	892.0
Organic matter	931.7
Crude protein	164.2
Ether extract	22.0
aNDFom	333.0
aADFom	187.9
Lignin (sa)	44.7
Lysine	7.8
Methionine+Cystine	7.5
Threonine	7.8
Calcium	9.6
Phosphorus	6.0
Digestible energy, MJ/kg[c]	9.9

[a]Contained>95% palygorskite [$(Mg,Al)_2Si_4O_{10}(OH)\cdot 4(H_2O)$] as agglomerant (binder).

[b]Premix provided per kg diet: vitamin A, 10,000 IU; vitamin D3, 1800 UI; vitamin E, 60 UI; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 0.02 mg; calcium pantothenate, 7 mg; nicotinic acid, 30 mg; folic acid, 0.5 mg; biotin, 0.2 mg; choline chloride, 400 mg; I, 1.5 mg; Mn, 60 mg; Cu, 6 mg; Zn, 80 mg; Fe, 30 mg; Co, 0.35 mg; antioxidant, 0.250 mg; 300 mg Cycostat (60 mg robenidine/kg). It did not contain any Se source (organic or inorganic).

[c]From tabulated data (FEDNA, 2003).

Animals, diets and experimental procedures

Ninety-six healthy 35-day-old weaned Hyla hybrid male animals were purchased from a breeding farm for meat rabbits. Handling and care of the experimental animals conformed to the guidelines of the Faculty of Animal Science and Aquaculture, Agricultural University of Athens. There were 4 dietary treatments of 24 rabbits each, which were fed either a basal pelleted diet (treatment BD, without any added Se) or the BD supplemented with Se from a yeast source, Sel-Plex® (Alltech Inc., Nicholasville, KY, USA), to provide additional 0.1, 0.5 and 2.5 mg Se/kg diet, in treatments LSe, MSe and HSe, respectively. The BD was formulated according to the recommendations of de Blas and Mateos (2010) for growing rabbits. The ingredients and chemical composition of the diets have been published elsewhere (Papadomichelakis *et al.,* 2017) and briefly outlined in Table 1.

Five days (72 d of age) prior to the end of the production cycle, 12 rabbits per treatment were randomly selected to collect blood and hair samples. One mL of blood was obtained from the ear artery (*Arteria auricularis*) with a sterilised syringe bearing an 18 gauge (1.27 mm outer nominal diameter) needle. Ear was cleaned with deionised water and acetone prior to blood sampling. Approximately 1 g of hair was cut with properly cleaned scissors (washed with deionised water and then with acetone) from the tail. Hair samples were washed with acetone, deionised water and then again with acetone to remove adherent dirt and organic materials. All tissue samples were stored at –20°C until analysis for trace element concentrations.

Determination of trace elements in tissues

Trace element concentrations in blood and hair samples were determined using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, Elan 9000; PerkinElmer Life and Analytical Sciences Inc., Waltham, MA, USA). ICP-MS can provide high-throughput, ultra-

trace level analysis down to ng/kg (Georgiou and Danezis, 2015). Complete digestion of the samples was performed with a microwave digestion system (Mars X-Press; CEM, NC, USA). Samples of whole blood (0.5 g) or hair (0.5 g) were soaked in 10 mL concentrated HNO_3 (65% w/v, Suprapur; Merck, Darmstadt, Germany). The samples were heated in the microwave-accelerated digestion system as follows: the power was ramped during 20 min from 100 to 1200 W and held for 15 min. The temperature reached a maximum of 200°C and was followed by a cool down cycle for 15 min.

Subsequently, samples were filtered with disposable syringe filters (Chromafil; Macherey-Nagel, Duren, Germany) and diluted 50 times with reverse osmosis water (Milli-Q Water Purification Systems, Billerica, MA, USA) prior to injection in the ICP-MS instrument. Standard solutions used for calibration curves were prepared from high-purity standards (Multi-element standard solution, Fluka Analytical; Sigma-Aldrich, St Louis, USA). The method and the instrumental parameters of the equipment have been described previously (Zoidis *et al.,* 2010). In brief, they were as follows: the nebuliser flow was 0.775 L/min, the vacuum pressure was 1.5×10^{-5} Torr, the lens voltage was 950 W, the analogue stage voltage was 1900 V, and the pulse stage voltage was 950 V. Twenty sweeps per reading were performed with one reading per replicate. The total number of replicates was 3. The total time per sample was 83 s. The analytical procedure was validated using standard reference material (NIST-RM 8414-bovine muscle powder; LGC Standards Promochem, Wesel, Germany) and a recovery procedure (Georgiou and Koupparis, 1990). The bovine muscle reference material was certified to contain 0.076±0.010 mg Se/kg, and the ICP-MS determined at 0.078±0.010 mg/Se kg. The recoveries of the procedure used to validate ICP-MS were in the range of 92.1-105.6%, indicating the accuracy of the method.

Statistical analysis

Data were analysed using the SPSS statistical package (version 17.0) and are presented as means±standard error of mean. Prior to analysis, data were tested for normality using Kolmogorov-Smirnov's test. The trace elements that were not normally distributed were transformed according to a 2-step approach that: i) transforms the variable into a percentile rank and ii) applies inverse-normal transformation to this rank, to form a variable consisting of normally distributed z-scores (Templeton, 2011).

Transformed data were analysed by a one-way (dietary Se level) analysis of variance (ANOVA). The linear and quadratic effects of dietary Se level were studied using polynomial contrasts. Pearson's correlation tests were used to determine significant inter-element correlations within the same tissue and correlation coefficients of elements between tissues. Discriminant analysis was also performed to investigate if the samples can be distinguished according to the dietary Se supplementation level, using blood or hair tissue trace element contents as predictor variables. Subsequently, a stepwise discriminant analysis aimed to establish those trace elements capable of distinguishing and classifying the samples. Wilks' lambda (λ) criterion was used to select the discriminant variables. Rabbit was the experimental unit and statistical significance was set at $P<0.05$ for all tests. For convenience, the means of the untransformed data are presented in the Tables.

RESULTS

The trace element concentration in the experimental diets is presented in Table 2. There were no differences between diets with the exception of Se level, which increased with increasing supplementation, as was expected.

Selenium content in blood and hair was affected linearly ($P<0.001$) by dietary Se inclusion. Diets supplemented with 0.5 and 2.5 mg Se/kg increased significantly ($P<0.001$) Se concentration by 145 and 290% in blood and by 201 and 500% in hair, in comparison with the basal diet. Supplementation with 0.1 mg Se/kg diet only increased the Se content of the examined tissues numerically (Tables 3 and 4). Selenium content appeared to be higher in hair than in blood. There was a linear decrease in Co ($P<0.001$), Cu ($P<0.001$), Mn ($P<0.05$), and Zn ($P<0.05$) concentration in blood with increasing Se supplementation, while Pb and Fe were not affected. On the other hand, there was a significant cubic effect of dietary Se on Co ($P<0.01$), Cu ($P<0.05$), Mn ($P<0.001$), Pb (<0.05), Mo ($P<0.05$) and Cd ($P<0.05$) levels in hair. Those elements appeared to increase in 0.5 mg Se/kg diet fed rabbits and then decrease in the 2.5 mg Se fed ones, when compared to the other two groups. Dietary Se supplementation linearly reduced

Table 2: Selenium (Se), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), antimony (Sb), arsenic (As), chromium (Cr), lead (Pb), molybdenum (Mo), nickel (Ni), zinc (Zn) and cadmium (Cd) concentrations (mg/kg as fed) in the experimental diets as determined by inductively coupled plasma mass spectrometry.

	Diet[a]			
Se added (mg/kg)[b]	0	0.1	0.5	2.5
Se	0.07±0.02	0.16±0.01	0.52±0.06	1.95±0.11
Co	0.74±0.09	0.80±0.07	0.74±0.08	0.79±0.03
Cu	18.09±2.58	18.39±1.83	17.89±0.97	17.36±0.44
Fe	610±52	623±37	581±64	597±61
Mn	139±6	141±16	132±5	135±7
Sb	0.023±0.003	0.027±0.001	0.026±0.001	0.022±0.001
As	0.005±0.001	0.005±0.002	0.004±0.003	0.004±0.001
Cr	0.082±0.006	0.085±0.010	0.080±0.008	0.081±0.011
Pb	0.186±0.006	0.197±0.006	0.186±0.003	0.179±0.004
Mo	1.20±0.06	1.16±0.06	1.13±0.27	1.10±0.09
Ni	7.55±0.07	7.60±0.42	7.38±0.17	7.38±0.21
Zn	125±17	125±18	126±18	117±3
Cd	0.125±0.007	0.124±0.005	0.121±0.010	0.121±0.011

[a]Average of 4 samples±standard deviation per diet.
[b]Diets were supplemented with Se from a yeast source, Sel-Plex® (Alltech Inc., Nicholasville, KY, USA).

Sb (P<0.001), As (P<0.001), Cr (P<0.001), Mo (P<0.001), Ni (P<0.001) and Cd (P<0.001) concentration in blood (Table 3). In hair, only As, Cr and Ni were decreased linearly (P<0.01, P<0.01 and P<0.001, respectively) with increasing dietary Se level. Overall, trace elements appeared to have higher concentrations in hair than in blood, with the exception of Fe and Ni (Tables 3 and 4).

Results show that Se was negatively correlated to Co, Cu, Mn, Sb, As, Cr, Mo, Ni and Cd (P<0.01) in blood. The highest correlation coefficients were observed between Se and Sb (0.712), As (0.889), Mo (0.743), Ni (0.729) and Cd (0.870) (Table 5). In hair, Se was negatively correlated to Fe (P<0.05), As (P<0.05), Cr (P<0.01), Pb (P<0.05) and Ni

Table 3: Selenium (Se), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), antimony (Sb), arsenic (As), chromium (Cr), lead (Pb), molybdenum (Mo), nickel (Ni), zinc (Zn) and cadmium (Cd) concentrations (µg/kg wet tissue) in blood, following dietary Se supplementation (Se) in rabbits at 72 d of age (means±SEM).

	Se supplementation (mg/kg)[a]				*P*-value[b]		
	0	0.1	0.5	2.5	L	Q	C
Se	157±6	177±5	228±5	455±19	<0.001	0.008	0.476
Co	12±2	23±11	7±0	7±0	<0.001	0.604	0.153
Cu	552±26	534±44	452±39	387±24	<0.001	0.677	0.773
Fe (×10^3)	412±11	413±10	406±8	420±8	0.847	0.146	0.752
Mn	58±20	79±36	16±5	24±8	0.011	0.726	0.150
Sb	2.0±0.5	2.0±0	1.0±0	1.0±0	<0.001	0.383	0.372
As	17±1	12±0	9.0±1	5.0±0.4	<0.001	0.733	0.237
Cr	24±1	24±3	17±2	14±1	<0.001	0.551	0.238
Pb	41±1	43±2	50±4	47±3	0.171	0.838	0.784
Mo	470±61	162±33	39±16	24±5	<0.001	0.018	0.178
Ni	2000±240	730±152	233±74	178±44	<0.001	0.028	0.216
Zn	2020±134	1970±76	1870±118	1670±79	0.019	0.411	0.800
Cd	58±3	43±4	30±2	15±2	<0.001	0.967	0.904

[a]Diets were supplemented with Se from a yeast source, Sel-Plex® (Alltech Inc., Nicholasville, KY, USA).
[b]Linear (L), quadratic (Q) and cubic (C) effects of dietary Se addition were studied by polynomial contrasts.

Table 4: Selenium (Se), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), antimony (Sb), arsenic (As), chromium (Cr), lead (Pb), molybdenum (Mo), nickel (Ni), zinc (Zn) and cadmium (Cd) concentrations (µg/kg wet tissue) in hair, following dietary Se supplementation (Se) in rabbits at 72 d of age (means±SEM).

	Se supplementation (mg/kg)[a]				*P*-value[b]		
	0	0.1	0.5	2.5	L	Q	C
Se	333±15	393±20	670±16	1664±42	<0.001	0.002	0.074
Co	44±7.5	33±3.1	50±6.1	32±2.2	0.016	0.553	0.005
Cu (×10^3)	12.1±0.4	11.9±0.2	12.9±0.4	12.3±0.3	0.062	0.284	0.016
Fe (×10^3)	11.4±0.1	8.7±0.9	55.4±28.1	12.7±5.7	0.016	0.163	0.006
Mn	538±71	463±30	692±51	540±50	0.025	0.048	<0.001
Sb	29±2.0	28±2.4	30±1.7	25±1.6	0.234	0.313	0.115
As	24±5.2	13±4.7	15±4.2	5.0±2.2	0.004	0.899	0.168
Cr	153±90	80±36	116±60	28±2.6	0.002	0.155	0.088
Pb	100±2.7	98±1.7	170±59	91±1.8	0.054	0.021	0.016
Mo	165±18	133±24	213±57	131±9.4	0.144	0.924	0.033
Ni	491±63	366±85	441±177	156±48	<0.001	0.177	0.177
Zn (×10^3)	256±20	254±5	273±5	265±5	0.003	0.102	0.084
Cd	10±2.6	7±1.4	29±4.1	14±2.0	0.032	0.057	<0.001

[a]Diets were supplemented with Se from a yeast source, Sel-Plex® (Alltech Inc., Nicholasville, KY, USA).
[b]Linear (L), quadratic (Q) and cubic (C) effects of dietary Se addition were studied by polynomial contrasts.

(*P*<0.01), and Zn was positively correlated to Se (*P*<0.01) (Table 5). In addition, the Se, As, Cr and Ni concentrations in blood were positively correlated to those in hair (*P*<0.001) (Table 6).

A discriminant analysis was additionally applied to blood and hair data to investigate whether the samples can be distinguished according to the dietary Se supplementation. Thirteen predictor variables (trace elements) were entered to develop 2 models to discriminate the analysed blood (*n*=48) and hair (n=48) samples. In blood, one discriminant function was statistically significant (*P*<0.001) for distinguishing the samples among the 4 dietary Se supplementation levels and described 95.6% of the observed variance. All 48 observations used to fit the model were classified into the correct group according to dietary Se supplementation. As shown in Figure 1, blood samples from the 4 groups were successfully separated from each other. A stepwise discriminant analysis revealed that mainly Se, followed by As and Mo, was responsible for the observed discrimination among the dietary Se supplementation levels in blood. In hair, two discriminant functions were statistically significant (*P*<0.001 and *P*<0.01, respectively) for distinguishing the samples among the 4 dietary Se supplementation levels and described 98.3% of the observed variance. Among the 48 observations used to fit the model, 97.2% were classified into the correct group according to dietary Se supplementation. As presented in Figure 2, hair samples from rabbits fed the supplemented diets with 0.5 and 2.5 mg Se/kg were successfully separated from those fed the basal diet and the diet supplemented with 0.1 mg Se/kg, which overlapped. Likewise, hair samples from rabbits fed the supplemented diet with 0.5 mg Se/kg were successfully separated from those fed

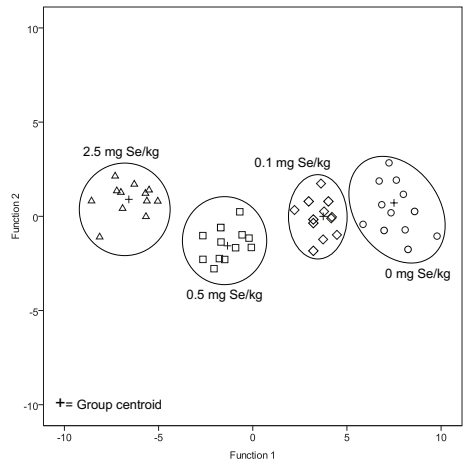

Figure 1: Discriminant plot separating the samples by the trace element concentration in blood according to dietary selenium (Se) supplementation in rabbits at 72 d of age (*n*= 12 rabbits/diet).

Table 5: Pearson correlation coefficients between trace element concentrations within blood and within hair of growing rabbits at 72 d of age.

Blood	Co	Cu	Fe	Mn	Sb	As	Cr	Pb	Mo	Ni	Zn	Cd
Se	-0.484**	-0.486**	0.230	-0.311*	-0.712**	-0.889**	-0.500**	0.123	-0.743**	-0.729**	-0.162	-0.870**
Co		0.456**	0.062	0.545**	0.339*	0.489**	0.674**	0.032	0.507**	0.584**	0.161	0.483**
Cu			0.086	0.264	0.451**	0.529**	0.571**	-0.150	0.504**	0.545**	0.230	0.507**
Fe				0.321*	-0.079	-0.094	0.137	-0.118	0.214	0.219	0.504**	-0.119
Mn					0.319*	0.364*	0.378**	0.001	0.301	0.264	0.175	0.410**
Sb						0.821**	0.385**	0.092	0.608**	0.629**	0.262	0.846**
As							0.533**	-0.107	0.810**	0.806**	0.203	0.942**
Cr								0.038	0.660**	0.670**	0.094	0.548**
Pb									-0.372*	-0.179	-0.238	-0.036
Mo										0.915**	0.446**	0.783**
Ni											0.390*	0.774**
Zn												0.152
Hair	Co	Cu	Fe	Mn	Sb	As	Cr	Pb	Mo	Ni	Zn	Cd
Se	-0.010	0.288	-0.323*	0.250	-0.085	-0.351*	-0.411**	-0.291*	-0.071	-0.601**	0.412**	0.207
Co		0.357*	0.489**	0.725**	0.342*	0.445**	0.625**	0.369*	0.577**	0.310	0.245	0.495**
Cu			0.268	0.470**	0.139	0.156	0.058	-0.059	-0.010	-0.030	0.507**	0.201
Fe				0.456**	0.593**	0.594**	0.341*	0.352*	0.292	0.462**	0.154	0.435**
Mn					0.255	0.291*	0.320*	0.205	0.371*	0.110	0.490**	0.514**
Sb						0.255	0.063	0.309*	0.123	0.100	0.305*	0.432**
As							0.338*	0.023	0.282	0.499**	0.132	0.204
Cr								0.408**	0.359*	0.534**	-0.109	0.255
Pb									0.255	0.353*	-0.111	0.269
Mo										0.482**	0.207	0.212
Ni											-0.009	0.152
Zn												0.364*

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Table 6: Trace element correlation coefficients between blood and hair of growing rabbits at 72 d of age.

Element	Coefficient	*P*-value
Se	0.822	<0.001
Co	0.012	0.937
Cu	-0.001	0.997
Fe	-0.181	0.223
Mn	-0.183	0.217
Sb	0.119	0.424
As	0.507	<0.001
Cr	0.491	<0.001
Pb	-0.083	0.581
Mo	0.251	0.134
Ni	0.621	<0.001
Zn	-0.045	0.763
Cd	-0.152	0.309

the 2.5 mg Se/kg diet. The stepwise analysis showed that Se was mainly responsible for the observed discrimination among the dietary Se supplementation levels in hair, followed by Cd and Cr.

DISCUSSION

The concentration of toxic or potentially toxic elements in animal tissues is of significance with regard to environmental pollution monitoring. It is also important in controlling the translocation of toxic elements through the food chain, particularly in those regions which may be exposed to contaminants due to dispersion and high levels of toxic metals in the environment (Rogowska *et al.,* 2009). Although meat rabbits are reared in closed buildings, the risk of exposure to toxic elements remains. The intake of plants with high mineral concentrations or the use of fertilisers, herbicides, insecticides and fungicides on crops, plants and/or grains which will be

used as feed is a potentially high risk (Reis *et al.,* 2010). Previous studies showed that a low, let alone a high intake of toxic elements, is reflected in the liver and muscle of meat rabbits (Papadomichelakis *et al.,* 2018).

In practice, edible tissue collection from meat animals can be performed only at the end of the fattening period. Hence, controlling changes in the toxic element contents throughout production becomes feasible when using alternative tissues like blood and/or hair, which do not require invasive or require minimally invasive methods and can be collected whenever needed. Hair has been used as a trace element biological indicator in the common European brown hare to study environmental pollution (Paukert and Obrusnik, 1986), but neither hair nor blood have previously been assessed in commercial rabbits. The present findings indicated that both tissues were responsive to dietary Se supplementation in a manner similar to that reported in earlier studies; Se concentration in blood and hair increased linearly with dietary Se addition, as has also been observed in the liver and muscle of the same rabbits (Papadomichelakis *et al.,* 2018). In addition, toxic (As and Cd) and potentially toxic (Sb, Cr, Ni, Mo and Ni) elements in blood decreased. Similar alterations were observed in hair, but for fewer elements; only As, Cr, Pb and Ni were affected by dietary Se. These differences can be solely attributed to dietary Se supplementation, as toxic elements occurred at similar levels in the experimental diets. The potential of dietary Se to reduce the levels of toxic and potentially toxic elements in blood and hair is partially in line with previous reports. Papadomichelakis *et al.* (2018) reported reduced contents of As, Cr, Ni and Cd in the liver, and also observed that As and Cd decreased in the muscle of rabbits fed Se supplemented diets. The dietary addition of Se compounds has been proven an efficient therapy against heavy metal toxicity in mammals (Glynn *et al.,* 1993), vegetables (Shanker *et al.,* 1996), poultry (Pappas *et al.,* 2011) and fish (Paulsson and Lundbergh, 1989). The current EU regulation limits the addition of organic Se to 0.2 mg/kg diet in all farm animals, so as not to exceed 0.5 mg of total Se/kg diet (EU, 2013). The addition of 0.5 and 2.5 mg organic Se/kg diet herein did not comply with the EU regulation. These dietary levels were used from a strictly scientific point of view and not as a practical consideration, in order to investigate whether the effects of Se excess would be reflected in blood and hair.

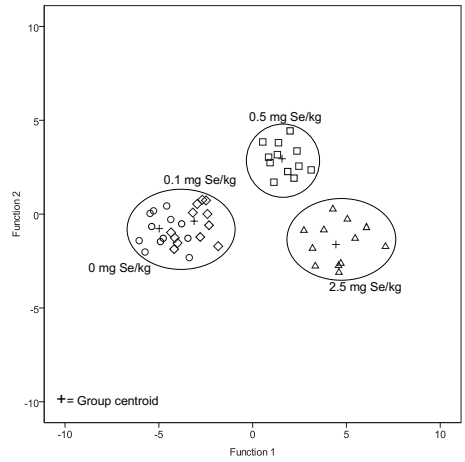

Figure 2: Discriminant plot separating the samples by the trace element concentration in hair according to dietary selenium (Se) supplementation in rabbits at 72 d of age (n=12 rabbits/diet).

The effectiveness of dietary Se against toxic elements in both tissues was also reflected in the correlation coefficients between trace element concentrations. Selenium was negatively correlated to almost all toxic and potentially toxic elements in blood and hair, which may be explained by the mode of action of Se. Selenium has been shown to lower the concentration of toxic elements by shifting the distribution of tissue elements from metallothioneins (MT) towards high-molecular-mass proteins (Underwood and Suttle, 1999; Wangher, 2001). Metallothioneins are low molecular weight cysteine-rich, intracellular proteins that bind metal ions for storage and/or for detoxification (Shen *et al.,* 2013). Selenium forms biologically inactive complexes with As (Levander, 1977), Cd (Ohta *et al.,* 1995) and Pb (Othman and El Missiry, 1998), which are excreted through bile, thus preventing accumulation in the body tissues of several animals (Glynn *et al.,* 1993; Pappas *et al.,* 2011; Paulsson and Lundbergh, 1989). Additionally, Se may participate in chelating Ni to proteins (Käkelä *et al.,* 1999) and may have antagonistic interactions with Cr (Soudani *et al.,* 2011), which likely explains the decrease in Ni and Cr concentrations, as well as the negative correlations between Se and Cr, and Ni in both rabbit tissues. Selenium was also negatively correlated to Pb in hair but not in blood. The hair consists of metabolically-dead material around the medulla and an active material only within the root. During hair growth, the active root cells are able to accumulate various elements depending on their concentration in the environment and in feed, as well as the time of exposure. As growing hair approaches the skin surface, it undergoes keratinisation and the trace elements accumulated during its formation are sealed into the keratin structure for long periods (Raab *et al.,*

2002; Hasan *et al.,* 2004). This evidence, along with the fact that approximately 8% of absorbed Pb is distributed to this tissue (Rogowska *et al.,* 2009), may explain why Se effects on Pb were evident in hair only.

Several other positive or negative correlations between trace element concentrations in blood and hair were observed, which highlight the complex nature of trace element interrelationships and will not be discussed in the present study. The aim was to investigate the suitability of blood and hair as biological trace element indicators. For this aspect, discriminant analysis proved useful. It was illustrated that the alterations in blood and hair trace element concentrations induced by dietary Se were sufficient to separate the four groups of rabbits; hence, both tissues appeared to be suitable as trace element indicators. However, the basal and the 0.1 mg Se/kg diet fed rabbits overlapped in the discriminant plot of hair. This may indicate that despite its suitability, hair cannot reveal small differences in trace element contents. On the other hand, these two groups of rabbits did not overlap in blood, which likely showed that blood may be a more sensitive biological indicator of trace element status. Hence, the effectiveness of dietary Se addition within the EU recommended levels (0.2 mg/kg; EU, 2013) against the accumulation of toxic and potentially toxic elements in growing rabbits was evident in blood, but not in hair. Additionally, blood appeared to indicate alterations in more trace elements when compared to hair, with the exception of Pb. This finding is in partial agreement with earlier studies in humans, where it was stated that Pb content in hair and blood could be used to characterise Pb exposure (Xing *et al.,* 2017). Moreover, there were significant correlations between the concentrations of some elements in different tissues; for instance, the contents of Se, As, Cr and Ni in hair were significantly positively correlated to those in blood. For Cd, an insignificant correlation was found between hair and blood concentrations, which suggested that hair Cd concentration is relatively independent of the Cd level in the blood, in agreement with earlier studies in cows (Rogowska *et al.,* 2009; Patra *et al.,* 2007). Although further research is necessary, the concentrations of the same elements in different tissues could indicate mutual reflectance and be complementary for assessment of corresponding heavy metal exposure, as was also suggested in human studies (Xing *et al.,* 2017).

In addition to the decrease in toxic and potentially toxic elements, dietary Se induced significant changes in the essential trace elements in blood and hair. The Co, Cu, Mn and Zn concentrations decreased linearly with increasing Se and were negatively correlated with Se in blood. It is known that Cu and Zn are joined in cellular defence against oxidants by Se to form a triad of trace elements that are involved in cytosolic antioxidant defence (Klotz *et al.,* 2003). There is a delicate balance between Se, Zn and Cu, in that the excess of one element may have an impact on the concentration of the others (Valko *et al.,* 2005) and this may explain the observed decrease in muscle Zn and Cu contents with increasing Se. The balance and the complex interrelationships between Se and other essential trace elements were also reflected in the reduced Co and Mn contents in blood. In hair, there was a different pattern of correlation between Se and other elements, which again highlights the need for complementary assessment of both tissues in exposure studies. Nevertheless, blood and hair appeared to be useful trace elements biological indicators in rabbits. Apart from heavy metal contents, they can provide additional information on the interrelationships between essential elements and between essential, toxic and potentially toxic elements.

CONCLUSIONS

In conclusion, alterations in the trace element concentrations induced by dietary Se supplementation were successfully reflected in blood and hair. Blood and hair can be used as non-invasive tissues to monitor toxic and potentially toxic substances, as well as essential trace element contents in meat producing rabbits. The differences in the levels of some elements between blood and hair indicate that these tissues should be used as complementary biological indicators in trace element studies.

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