Journal of Animal and Feed Sciences, 27, 2018, 307–316 *https://doi.org/10.22358/jafs/99863/2018*
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Influence of soil contamination before and after ensiling on mineral composition of grass silages, feed intake and carry-over to body tissue of goats

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KEY WORDS: tissues, goats, grass silage, iron, soil, trace elements

Received: 4 December 2017
Revised: 12 September 2018
Accepted: 11 December 2018

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ABSTRACT. Iron (Fe) is ubiquitous in the environment and has possible impact on quality and safety of feed and food due to the fact that it can be transferred from soil to animal feed and further to the products of animal origin. Therefore, the objective of the present study was to evaluate the effect of contamination of forage with soil differing in Fe concentration on Fe solubility, mineral composition and quality of grass silages. Furthermore, the effect of feeding these silages on feed intake, performance, trace element absorbability and carry-over into edible tissues was tested in young goats. Two ensiling experiments revealed that treating grass without or with different levels and types of soil before ensiling did not affect fermentation parameters of silages. Nevertheless, the addition of soil caused a highly significant increase of crude ash and trace elements contents in forage and silages. During ensiling, the *in vitro* solubility of Fe increased on average 5 times. Also, the aluminium content was the best indicator of soil contamination in forages. In a feeding trial with growing goats, feed intake and live weight gain were decreased in the group fed grass ensiled with the soil in contrast to the control group and animals receiving feed with soil added just before feeding. Fe concentration was highest in the duodenal tissue of kids fed the forage contaminated with soil before ensiling (184 vs 88–80 mg Fe/kg DM in the other two treatments), which might be also an indicator of Fe regulating properties. Fe concentration in the liver increased likewise. In conclusion, data indicate that ingesting ensiled soil impairs animal performance.

Introduction

Iron (Fe) is a ubiquitous element. It is present also in the rumen of cattle, sheep and goats. Drinking water, feed naturally rich in Fe, phosphate supplements and forage products from grassland as well as from abrasion from agricultural machinery are relevant sources of Fe (Hansen and Spears, 2009). The recommendations of daily Fe supplementation in cattle and goats are rather similar and amount to 50 mg and 40–50 mg per kg dietary dry matter (DM), respectively (GfE, 2001, 2003; NRC, 2001; The European Union (EU) Regulation (EG) No. 1334/2003 (EU, 2003) allows a maximum Fe level

of 750 mg/kg (at 88% DM) as a feed additive in complete feed, while the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered 450 mg/kg DM as safe for bovines (EFSA, 2016). For sheep, a maximum tolerable level in the EU is 500 mg/kg feed DM (EFSA, 2016).

On the other hand, after a long-term monitoring (years 2000–2014) of cattle feed in Saxony it was revealed that a great part of analysed grass (n = 394) and lucerne (n = 167) silages plus a small percentage of total mixed rations (n = 1733) exceeded the threshold of 450 mg/kg (Steinhöfel et al., 2016).

In general, Fe is present in soil mostly in an insoluble form characterized by a low absorbability. The non-ionic form of Fe passes the intestine without harming health and productivity of animals (Hansen and Spears, 2009). However, Healy (1972) demonstrated that in gastrointestinal fluids of ruminants Fe compounds from soil may be partly soluble. The released Fe ions can be absorbed or affect the mineral composition of the digesta. They may also have antagonistic effects – by binding with other elements they reduce their solubility and absorption. The solubility of Fe compounds is enhanced in an acidic environment (Hansen and Spears, 2009). An increased absorbability of Fe in forages contaminated with soil has been described and may be due to a reduction of ferric Fe to ferrous Fe during ensiling (Whitehead, 2000).

Animals are able to control their Fe metabolism strictly by homeostatic mechanisms. Free Fe is highly reactive and thus toxic, but on the other hand (pathogenic) bacteria can grow faster in Fe abundant environments (Ganz, 2003). Hepcidin has been identified as a key hormone stimulating Fe absorption especially in regular and suboptimal Fe supply conditions (Ganz, 2003; Enculescu et al., 2017). Furthermore, also hepcidin-independent mechanisms are discussed, especially during excess Fe supply, called dietary uptake saturation (Enculescu et al., 2017). However, despite the indicated mechanisms of Fe homeostasis, an excess Fe supply of cattle and sheep has been described to decrease feed intake and having antagonistic effects to other trace elements such as Cu, Zn and Mn. Furthermore, Fe excess causes poor appetite, decreased feed utilization and decreased body weight gain (Standish et al., 1969; Flachowsky et al., 1976; Grün et al., 1978; Lamand et al., 1979; Suttle, 2010).

Therefore, in this study it was hypothesized that the contamination of forage with mineral soil from different origins increases the Fe content and impairs fermentation quality of grass silages. Furthermore, it was expected that ensiling increases Fe solubility and thus absorbability in the animal's gastrointestinal tract. Other analytical parameters than crude ash can better differentiate mineral content from forage vs soil. It was also tested if the time of forage contamination with soil (before and after ensiling) affects animal performance and accumulation of trace elements in tissues of goats.

Material and methods

Quality and mineral composition of laboratory scale silages (Trial 1)

Grass silages were prepared from forage harvested on July 1, 2014, from semi-intensive grassland in Northern Saxony (Germany), and wilted to 25 or 45% DM. Grass was chopped by an electric garden shredder (AXT 2200, Bosch, Stuttgart, Germany) to a theoretical length of 3 cm. Mineral soil of varying Fe content (23 200 and 48 700 mg Fe/kg DM, Fe_{Low} and Fe_{High}, respectively) from two locations in Saxony was dried at 65 °C and milled using a Pulverisette 14 (Fritsch, Idar-Oberstein, Germany) (2.0 mm sieve) to achieve high homogeneity. Each of the two wilting levels were treated as follows: no contamination with soil (Control), low contamination with soil (Fe_{Low}) and high contamination with soil (Fe_{High}), both at 100 g/kg forage DM, resulting in six treatments in total. Beside iron, the soils Fe_{Low} and Fe_{High} contained 56 500 and 76 500 mg Al/kg DM, respectively, as well as 372 000 and 316 000 mg Si/kg DM, respectively. The content of Zn, Mn and Cu was slightly higher in soil Fe_{High} than in soil Fe_{Low}.

For ensiling at laboratory scale, 1.5-l preserving jars with spring clips and rubber bands (Weck®, Wehr, Germany) were used as experimental silos (three silos per treatment). The forage was thoroughly mixed with soil by hand. Each silo was tightly packed with 650 g forage fresh matter independent of wilting level. Control treatments without soil addition were also ensiled at both wilting levels. All treatments were left to ferment for 60 days. Thereafter, silos were opened and the pH was measured immediately and sensory evaluation was carried out according to the German Agricultural Society (Deutsche Landwirtschafts-Gesellschaft – DLG) guideline (DLG, 2004). Subsamples underwent an aerobic stability test according to Honig (1990), in which temperature rise against ambient temperature was measured during 6.25 days of aerobic storage. The remaining amount of silages was stored at −20 °C until further analysis.

Chemical composition of forage and silages was determined at the Saxon State Company for Environment and Agriculture (BfUL) according to standard methods (VDLUFA, 1976). The content of the following components was analysed: DM, crude ash, crude protein, crude fibre, sugar (Luff-Schoorl method), ADFom and aNDFom [acid detergent fibre (ADF) and neutral detergent fibre (NDF) expressed on organic matter basis] as well as ammonium. HCl insoluble ash was determined according to the Association of German Agricultural Analytic and Research Institutes (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten VDLUFA) (VDLUFA, 1997). Mineral composition was determined in soils, forages and silages by means of S8 Tiger apparatus (Bruker, Karlsruhe, Germany) with X-Ray fluorescence. The dietary cation-anion difference (DCAD) was calculated (Ender et al., 1971). Ensiling quality was evaluated chemically (DLG, 2006) based on pH values related to DM content and content of butyric acids (sum of C4:0–C6:0 acids: n-butyric, isobutyric, n-valeric, isovaleric, n-caproic) and acetic acid (sum of C2:0–C3:0 acids: acetic, propionic) analysed with Reversed Phase-HPLC (LC-20A Prominence, Shimadzu Deutschland GmbH, Duisburg, Germany; column Hi-Plex H 8 µm, 300 × 7.7 mm, Agilent, Santa Clara, CA, USA).

Data obtained in the study were subjected to a two-way analysis of variance by STATISTICA version 10 (StatSoft PL, Kraków, Poland) and the significance of differences was estimated by Duncan's test at $\alpha = 0.05$. The effects of DM and soil addition and their possible interactions were tested.

Ensiling trial to test Fe solubility before and after ensiling (Trial 2)

In a separate ensiling experiment, carried out in May 2010 in Middle Saxony (Germany), Italian ryegrass (*Lolium multiflorum*) was cut, wilted and chopped to 3 cm theoretical length by a forage harvester. A chemical silage additive containing hexamine and $NaNO_2$ was applied at 2 ml per kg fresh matter (FM). Mineral soil from ten different farm locations with Fe contents ranging from 1 600 to 11 300 mg/kg DM, prepared as described above, was added at 40 or 80 g per kg forage DM, respectively, and mixed thoroughly. The resulting 20 treatments were ensiled in triplicate in 2.5-l Weck® jars (around 1200–1400 g FM per jar) for 96 days. Fermentation quality was assessed by sensory evaluation. Crude ash and Fe contents were determined before and after ensiling, the latter by atomic absorption spectroscopy at the Freie Universität Berlin (Germany). As indicator for bioaccessibility, Fe solubility before and after ensiling was assessed, following a method adapted from Hansen and Spears (2009). Briefly, different forage treatments before ensiling and silages were freeze dried and milled by a centrifugal mill (Retsch, Haan, Germany) to 0.5 mm. The freeze dried material (1 g) was mixed with 40 ml distilled water and extracted at a shaking rate of 80/min for 5 h in a laboratory shaker. The supernatant was then filtered through an ash free filter. The total content of Fe and the Fe content in the supernatant (soluble part) were analysed according to Annex IV, C (Commission of the European Communities, 2009).

The data were analysed by descriptive statistics. The effects of ensiling and the added soil level were tested by the Univariate procedure using SPSS® Statistics version 19 (IBM®, Armonk, NY, USA) applying a significance level of $\alpha = 0.05$.

Silage preparation for the feeding experiment

Italian ryegrass was cut on a farm in the Ore Mountains (Germany) in July 2010. The grass was wilted (targeted at 300 g/kg, final DM about 405 g/kg). It was chopped to a nominal length of 40 mm and baled (John Deere 744 Premium Wrapping Baler (Deere & Company, Arc-lès-Gray, France) with a MaxiCut 25-knife fine chop pre-cutter) to produce 8 wrapped bales of silage [used for control and $Soil_{post}$ (contaminated after ensiling) groups] to give a total of 3.12 t DM. For the $Soil_{pre}$ treatment (contaminated before ensiling), 14 g fresh topsoil from the farm's cropland was applied by hand per kg wilted forage FM on the swath. The soil contained 717 g crude ash, 8300 mg Fe, 11 mg Zn, 134 mg Mn and 6 mg Cu per kg DM. The swath was taken up, chopped, baled and wrapped in four bales accordingly to give a total of 1.25 t DM.

The $Soil_{post}$ treatment was prepared using dried soil from the same site as for $Soil_{pre}$, aiming at a similar final Fe content of the diet.

Feeding experiment

The trial comprised three feeding groups of eight growing animals each (male goats, German goat breed 'Weisse Deutsche Edelziege'). Due to the time needed to get the approval from the Ethical commission (Reg 0331/10, Landesamt für

Gesundheit und Soziales Berlin) it was carried out only in 2012. At the start of the experiment, the animals were 6 weeks old and had an average live weight of 11.8 ± 2.8 kg. Two animals per unit were kept together to assess the feed intake. The period to adapt to grass silage as sole feed component lasted 14 days. The trial was conducted on the experimental station of the Federal Institute for Risk Assessment (BfR) in Berlin (Germany) and lasted 85 days. The animals were assigned to three treatments: control (grass silage without soil contamination), $Soil_{pre}$ (grass silage contaminated with soil before ensiling) and $Soil_{post}$ (grass silage contaminated with soil at 130 g/kg DM after ensiling just before feeding).

The goats were fed the respective grass silages once a day *ad libitum*, i.e. depending on their foregoing intake they were fed around 200–300 g DM on day 8 which was increased to 480–740 g DM on day 85 of the experiment. To achieve a live weight gain of 50 g/day a male kid of 15 kg needs around 4.5 MJ ME/day, and 5.7 MJ ME with 20 kg live weight which corresponds to about 410 and 520 g DM at 11 MJ ME/kg DM (GfE et al., 2003). During the experiment, animals were weighed weekly to calculate the daily weight gain. Feed left-overs were recorded daily. Feed samples were taken daily, frozen and pooled for analysis of crude ash and trace elements. All 24 goats from the experiment were slaughtered after experimental feeding. At slaughtering, samples were taken from the back muscle (*Musculus longissimus dorsi*), from the ham (*Musculus glutaeus maximus)*, the liver, the kidneys and the duodenal tissue. Animal tissue and feed samples were analysed for Fe, Zn, Cu and Mn by atomic absorption spectroscopy (contra®AA 700, Analytik Jena AG, Jena, Germany) in the laboratory of the Institute for Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin (Germany). The samples were prepared by freeze drying, milling and incineration. The crude ash was treated with concentrated HCl and distilled water before heating in a sand bath and filtering through a folded filter.

Data obtained in the feeding trial were subjected to the procedure Univariate by SPSS® Statistics ver. 19 (IBM®, Armonk, NY, USA) evaluating the treatment effect (for the analysis of the tissue also the effect of the type of tissue and a possible interaction between type of tissue and treatment) and Tukey-HSD post hoc test at a significance level of $\alpha = 0.05$.

Results

Quality and mineral composition of laboratory scale silages (Trial 1)

The chemical composition of silages contaminated with soil or not is presented in Table 1. The content of crude ash was high (> 100 g/kg DM) in all silages and exceeded 170 g/kg DM when treated with soil ($P < 0.001$). Contents of manganese and all other analysed trace elements in silages were significantly ($P < 0.01$) higher at 25% DM compared to 45% DM. In soil contaminated silages, the increase of acid insoluble ash was even more pronounced and reached about the 2.5 fold over the control. The soil contamination caused a reduction of crude protein content by about 5 to 20 g/kg DM ($P < 0.05$) and of the fibre fractions (ADFom and aNDFom) by about 25 g/kg ($P < 0.05$). Macro-elements like sodium, chloride and potassium were not distinctively affected by treatments, only the sulphur content was decreased ($P < 0.05$) and the Mg content was increased ($P < 0.001$). These changes did not affect the Dietary Cation-Anion Difference (DCAD) of silages, which was similar between the treatments (Table 1). As expected, the addition of soil caused a highly significant ($P < 0.001$) increase of Fe (up to 34 fold), but also of manganese (4 fold), silicon (2 fold) and aluminium (80–100 fold) contents, especially in Fe_{High} treatment. There were no consistent differences in mineral concentration in the experimental silages in comparison to the corresponding initial materials (data not shown). In general, the ensiling quality of all the silages was good (Table 1) and only the DM level affected the pH value. The contamination with soil did not change the measured parameters significantly. Despite some differences in butyric acid contents among DM levels, there was no indication of strong butyric acid fermentation (butyric acid < 10 g/kg DM).

The ammonia nitrogen concentration as indicator for deamination was similar in all silages and slightly above the critical value of 80 g/kg N. All silages were aerobically stable for > 4 days, surpassing the critical first 3 days.

Ensiling trial to test Fe solubility before and after ensiling (Trial 2) (Trial 2)

The addition of two levels of soil from 10 different origins resulted in Fe levels ranging from 531 to 3–714 mg/kg DM with significant differences caused by soil level (Table 2). Before ensiling, about 7% of the Fe was soluble

Table 1. Chemical composition of silages, g/kg dry matter (DM) (if not stated differently) (Trial 1)

Indices	DM in forage, %		Treatments[1]			*P*-value			SEM
	25	45	Control	Fe_{Low}	Fe_{High}	DM	Soil	DM × Soil	
Nutrients									
DM, g/kg	266.5^{B}	481.7^{A}	358.7	376.3	400.8	< 0.001	0.621	1.000	27.2
Crude ash	159.8^{A}	148.5^{B}	114.3^{B}	171.7^{A}	172.5^{A}	0.001	< 0.001	0.244	6.7
Acid insoluble ash	74.3^{A}	66.9^{B}	33.9^{B}	90.3^{A}	84.6^{A}	0.001	< 0.001	0.123	6.1
Crude protein*	161.5^{B}	177.3^{A}	186.0^{Aa}	171.2^{b}	165.7^{B}	0.009	0.022	0.840	3.5
Crude fibre*	237.4	230.8	250.48^{A}	222.6^{B}	225.6^{B}	0.249	0.005	0.031	4.2
ADFom*	249.5	245.0	264.5^{A}	236.0^{B}	236.7^{B}	0.536	0.006	0.888	4.5
aNDFom*	471.0	479.3	506.4^{A}	465.1^{B}	462.3^{B}	0.536	0.023	0.734	7.5
Minerals									
DCAD, meq/kg DM	308	324	316	312	320	0.300	0.933	0.926	6.5
Na	0.4^{a}	0.3^{b}	0.31^{b}	0.44^{a}	0.37^{ab}	0.048	0.034	0.998	0.0
K	30.0	29.6	30.6	29.4	29.5	0.408	0.053	0.734	0.2
S	3.4	3.3	3.5^{a}	3.3^{b}	3.3^{b}	0.463	0.016	0.657	0.0
C	9.5	8.6	9.4	9.1	8.7	0.179	0.734	0.965	0.3
Ca	6.9^{a}	6.4^{b}	6.8	6.6	6.6	0.020	0.621	0.589	0.1
P	3.6^{A}	3.3^{B}	3.5	3.4	3.4	0.001	0.640	0.904	0.0
Mg	1.9^{A}	1.8^{B}	1.7^{C}	1.8^{B}	2.0^{A}	< 0.001	< 0.001	0.018	0.0
Fe, mg/kg DM	2099^{A}	1707^{B}	235^{C}	1813^{B}	3381^{A}	< 0.001	< 0.001	< 0.001	321
Mn, mg/kg DM	84^{A}	74^{B}	34^{C}	92^{B}	106^{A}	0.001	< 0.001	0.067	8
Zn, mg/kg DM	45^{A}	42^{B}	40^{B}	41^{B}	49^{A}	0.005	< 0.001	0.076	1
Cu, mg/kg DM	9.5^{A}	8.8^{B}	8.2^{B}	8.4^{B}	10.5^{A}	0.001	< 0.001	0.011	0
Si, g/kg	34.6^{A}	31.7^{B}	25.5^{B}	37.2^{A}	36.0^{A}	0.003	< 0.001	0.287	1.3
Al, mg/kg DM	2802^{A}	2198^{B}	60^{C}	2764^{B}	4322^{A}	< 0.001	< 0.001	0.002	439
Fermentation parameters									
Storage losses, % FM	1.0	1.0	1.1	1.0	0.9	0.660	0.485	0.703	0.1
Aerobic stability, h	131	141	100	143	150	0.515	0.110	0.317	9
NH_3-N, % total N	9.6	9.2	9.8	9.3	9.2	0.498	0.714	0.920	0.3
pH	4.06^{B}	4.72^{A}	4.50	4.36	4.37	< 0.001	0.243	0.931	0.1
Butyric acid	0.00^{B}	0.91^{A}	0.5	0.5	0.5	0.007	0.958	0.958	0.2
Acetic acid	7.3	7.5	8.1	6.9	7.3	0.644	0.113	0.001	0.3
Ethanol	0.7	0.7	0.8	0.6	0.6	0.882	0.146	0.781	0.0
DLG sensory quality[2]	2.0	3.9	1.8	3.8	3.2	0.156	0.406	0.349	0.6
Ensiling quality[3]	95.6^{B}	98.5^{A}	95.0^{B}	97.5^{A}	98.6^{A}	< 0.001	< 0.001	0.006	0.6

ADFom – acid detergent fibre expressed exclusive of residual ash, aNDFom – neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash, DCAD dietary cation-anion difference; [1] treatments: Control – forage crop without soil contamination, Fe_{Low} – forage contamination with soil of low Fe content, Fe_{High} – forage contamination with soil of high Fe content; [2] DLG sensory quality: marks from 1 to 5 (very good to very poor); [3] ensiling quality points based on chemical analysis: up to 100 (best quality); * determined from forage before ensiling; ^{ab} – means within the same row and treatment not sharing the same superscript are significantly different at $P < 0.05$; ^{ABC} – means within the same row and treatment not sharing the same superscript are significantly different at $P < 0.01$

Table 2. Contents of crude ash and Fe (means of fresh and ensiled forage) and Fe solubility before (fresh) and after ensiling (Trial 2)

	Added soil per kg forage dry matter (DM)						Overall mean	SD	*P*-value	
	+ 40 g			+ 80 g					ensiling	soil level
	mean	min	max	mean	min	max				
Crude ash, g/kg DM	126	119	134	148	137	166	136	14.8	0.818	< 0.001
Fe, mg/kg DM	898	531	1541	1606	812	3714	1254	733.0	0.225	< 0.001
Fe solubility, %										
Fresh	7.00	4.76	11.09	6.61	4.04	13.19	6.81	2.273	–	0.718
Ensiled	36.32	20.30	45.90	32.39	15.80	46.23	34.42	7.177	< 0.001	0.036

SD – standard deviation

in water. After ensiling, the Fe solubility raised to 19–42%, which means on average a 4-fold increase ($P < 0.001$).

Feeding experiment

In the silages contaminated with soil, the Fe concentration was 5- to 6-fold higher than in the control silage and more than two times higher than acceptable daily allowance for bovine and ovine (Table 3). The Fe content in the feed was numerically the highest in the $Soil_{post}$ treatment, where soil was added after ensiling just before feeding (Table 3).

During the feeding trial, animals from $Soil_{post}$ group showed the highest daily Fe intake (Table 4) as a consequence of higher feed intake. The daily feed intake was the lowest in $Soil_{pre}$ group and the highest in $Soil_{post}$ group, however the control group did not differ from neither $Soil_{pre}$ or $Soil_{post}$.

Table 3. Nutrient composition and contents of Fe, Cu, Zn, Mn of baled grass silages (or forages at ensiling*) (Feeding trial)

Indices	Treatment[1]		
	Control (no contamination)	$Soil_{pre}$	$Soil_{post}$
DM, g/kg	298	317	336
Nutrients, g/kg DM			
Crude ash	120	157	173
Crude protein	193	193	
Crude fibre	280	249	
Ether extract	51	51	
Sugar*	149	136	
ADFom*	232	238	
Ca*	4.79	4.77	
P*	3.41	3.50	
Na*	0.20	0.23	
Mg*	2.13	2.23	
S*	2.45	2.44	
Cl*	3.51	3.29	
Si*	n.a.	11.30	
mg/kg DM			
Al*	n.a.	1650	
Fe	350	1713	2064
Zn	27	30	42
Mn	26	58	69
Cu	8	8	9
NEL, MJ/ kg DM*	6.9	6.7	
ME, MJ/kg DM*	11.2	11.0	

[1] Treatments: Control – without soil contamination, $Soil_{pre}$ – soil contamination before ensiling, $Soil_{post}$ – soil contamination after ensiling, just before feeding; n.a. – not analysed; NEL – net energy for lactation; ME – metabolizable energy (GfE, 1995)

The daily live weight gain (LWG) of $Soil_{pre}$ group was the lowest and was about half of the other two experimental groups (Table 4). Between treatments the weekly live weight development was clearly distinguished at the end of the feeding period at day 86 with the lowest live weight in $Soil_{pre}$ group ($P = 0.048$, Figure 1). Correspondingly, the feed conversion ratio was most limited in $Soil_{pre}$ group (Table 4).

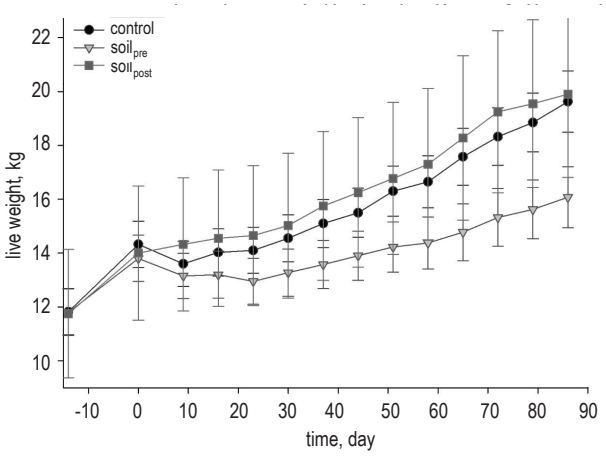

Figure 1. Live weight development of animals fed one of the treatments: control – without soil contamination, $Soil_{pre}$ – soil contamination before ensiling, $Soil_{post}$ – soil contamination after ensiling, just before feeding (means, error bars indicate standard error of the mean, n = 8)

Table 4. Dry matter intake (DMI) and Fe intake per animal per day and growth performance from experimental days 8 to 85, feeding trial (means ± standard deviation)

Indices	Treatment[1]			*P*-value
	Control (n = 4)	$Soil_{pre}$ (n = 4)	$Soil_{post}$ (n = 4)	
Daily DMI, g/animal	$387^{ab} \pm 52.3$	$312^{b} \pm 43.8$	$438^{a} \pm 30.9$	0.006
Daily Fe intake, g/animal	$135^{c} \pm 18.2$	$534^{b} \pm 75.1$	$874^{a} \pm 95.5$	< 0.001
LW, kg (n = 8)				
day 0	14.3 ± 2.3	13.8 ± 2.5	14.0 ± 2.5	0.91
day 85	19.6 ± 3.5	16.1 ± 3.0	19.9 ± 3.1	0.048
Daily LWG, g/animal	78^{a}	38^{b}	72^{a}	0.002
Feed conversion, kg DMI/kg LWG	5.0^{b}	9.0^{a}	5.9^{b}	0.012

[1] Treatments: Control – without soil contamination, $Soil_{pre}$ – soil contamination before ensiling, $Soil_{post}$ – soil contamination after ensiling, just before feeding; DMI dry matter intake; LW – live weight; LWG – live weight gain; ^{abc} – values with different superscripts within a row are significantly different at $P < 0.05$ (Tukey-HSD)

by the kidneys and the duodenal tissue where significant differences between treatments were observed (Table 5). However, despite the low level, the Fe content in tissues of $Soil_{pre}$ group was significantly higher than for the other two treatments even in the

ham. Only the *Musculus longissimus dorsi* showed no response to treatment in none of the analysed parameters. Copper also accumulated mostly in the liver (10-fold more in comparison to muscle meat), however it was not influenced by the treatments (Table 5). The zinc content was the highest in ham and liver. Elevated values in these tissues and in the intestinal wall were noted in $Soil_{pre}$ group. The liver was also a main deposit for manganese, the muscle tissues contained only one tenth of this concentration (Table 5).

Table 5. Contents of dry matter (DM), crude ash and trace elements in tissues of goats after 86 d of experimental feeding (Feeding trial; n = 8)

Indices	DM, g/kg	Crude ash, g/kg DM	Trace elements, mg/kg DM			
			Fe	Zn	Cu	Mn
Musculus longissimus dorsi						
Control[1]	207.7	44.2	40	80	4	0.3
$Soil_{pre}$ [1]	206.4	46.0	36	78	3	0.3
$Soil_{post}$ [1]	209.5	42.0	39	71	3	0.2
SEM	1.92	0.77	2.0	2.2	0.2	0.02
P-value	0.809	0.131	0.671	0.248	0.208	0.187
Musculus glutaeus maximus						
Control	150.4	52.2	39^{b}	108^{b}	2.6	0.2^{a}
$Soil_{pre}$	148.8	51.3	57^{a}	148^{a}	4.5	0.4^{b}
$Soil_{post}$	147.3	51.4	44^{b}	133^{ab}	3.1	0.3^{ab}
SEM	1.03	0.45	1.9	6.0	0.4	0.02
P-value	0.490	0.692	0.005	0.044	0.099	0.031
Liver						
Control	207.7	49.7	298^{b}	93	23	4.3
$Soil_{pre}$	213.9	49.8	574^{a}	151	77	4.0
$Soil_{post}$	210.1	49.1	426^{ab}	95	22	4.6
SEM	3.59	0.50	28.8	23.9	11.3	0.12
P-value	0.768	0.866	0.003	0.529	0.102	0.139
Kidneys						
Control	183.4	64.9	193^{b}	70	14	1.7
$Soil_{pre}$	184.2	63.4	290^{a}	70	14	1.5
$Soil_{post}$	185.3	63.0	203^{b}	71	14	1.8
SEM	1.45	0.82	7.11	1.0	0.3	0.04
P-value	0.864	0.612	< 0.001	0.787	0.877	0.064
Duodenal tissue						
Control	208.7^{b}	62.7	88^{b}	57^{b}	3.3	1.34
$Soil_{pre}$	175.7^{a}	68.9	184^{a}	69^{a}	4.1	2.71
$Soil_{post}$	211.5^{b}	54.7	80^{b}	53^{b}	3.3	1.38
SEM	5.41	2.53	8.6	1.8	0.2	0.27
P-value	0.023	0.102	< 0.001	0.004	0.225	0.076
P-value (overall)						
Body tissue	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment	0.092	0.021	< 0.001	0.146	0.060	0.343
Body tissue × treatment	0.004	0.068	< 0.001	0.735	0.013	0.002

[1] Treatments: Control – without soil contamination, $Soil_{pre}$ – soil contamination before ensiling, $Soil_{post}$ – soil contamination after ensiling, just before feeding; SEM – standard error of the mean; [ab] – the values with different superscripts within each column (for each tissue separately) are significantly different at $P < 0.05$

Discussion

Analytic indicators of soil contamination

In feedstuffs, the crude ash content is a sum parameter for minerals. In grass silages, a content of > 100 g/kg DM is usually undesired as it is commonly assumed to originate from soil contamination, which can dilute nutrients like crude protein and displace fibre components in the roughage. However, the presence of crude ash *per se* does not permit to clearly indicate the origin of minerals. Some pastures, especially when rich in herbs, can contain substantial amounts of plants' own minerals such as calcium. Thus, acid insoluble ash is recognized as parameter for the sand proportion (VDLUFA, 1997). The inclusion of 100 g soil/kg forage DM increased more than two times the concentration of acid insoluble ash (Table 1). The analysis of soil composition revealed that silicon and aluminium are the most and the second most abundant elements in mineral soils, respectively. However, the most pronounced difference of all analysed parameters in the silages without and with soil contamination can be found in the aluminium concentration (Table 1), which was from 46- to 72-fold higher when soil was added, while silicon increased by only 0.40 to 0.45 times. Thus, in the context of this experiment the aluminium content is an important indicator of soil contamination in forages.

Ensiling soil contaminated forage

The Fe content in grass silages was strongly influenced by the degree of soil contamination and soil origin. The higher concentration of trace elements at lower DM content can be probably explained by the better adherence of soil to wetter forage. Soil contamination slightly decreased nutrient concentration of main nutrients such as crude protein. However, major mineral elements were not affected; therefore the DCAD was not changed by the treatments. In the Trial 2, ensiling increased the solubility of Fe from 7 to 34% on average for fresh and ensiled grass, respectively. This is not as high as observed with whole crop maize (Hansen and Spears, 2009). Nevertheless, a clear increase in bioaccessibility of Fe can be assumed. Ensiling did not change the concentration of minerals unlike often observed in practice. That might be due to a low loss of organic matter in small experimental silos. Soil contamination is seen as one factor to provoke clostridial fermentation (Pahlow et al., 2003). However, in our case, fermentation quality was not altered negatively. In the Trial 2, the added

$NaNO_2$ might have helped to counteract clostridial activity (Kaiser and Weiss, 2007). However, in Trial 1 no additive was applied. Optimal ensiling conditions at laboratory scale might have triggered the lactic acid fermentation with a rapid pH decline thus suppressing butyric acid bacteria.

Feeding of soil contaminated silage

The higher Fe content in $Soil_{post}$ treatment in comparison to $Soil_{pre}$ treatment might be explained by field losses of soil during baling and its higher moisture content at ensiling. Despite a nearly 4- to 6-fold higher Fe intake by $Soil_{pre}$ and $Soil_{post}$ groups, the Fe content in the liver of the goats was only doubled as compared to the control. This corresponds to findings of Enculescu et al. (2017) who stated that Fe pools do not respond to dietary changes in a proportional manner. The uptake of Fe from the intestinal lumen into duodenal enterocytes *via* the divalent metal Fe transporter 1 (DMT1) is regulated locally by cellular Fe levels and increased by the presence of reducing substances such as different proteins in the arterial blood (Kirchgessner et al., 2008; Hansen et al., 2010). In conditions of excess Fe supply, Fe transporters are downregulated by Fe regulatory proteins/Fe responsive elements to decrease Fe absorption (Galy et al., 2013). Thus, it seems to be reasonable that the highest relative increase in Fe concentration was found in the duodenal tissue of $Soil_{pre}$ group, followed by the liver. This observation confirms, at the same time, that more absorbable Fe was present than in $Soil_{post}$ treatment as described by Whitehead (2000) and Hansen and Spears (2009) and as shown by the increased Fe solubility in the ensiling trial. The enhanced Fe content in the intestinal tissue of $Soil_{pre}$ group in our study is in contrast to a study on calves by Hansen et al. (2010), in which Fe content remained stable when Fe was supplemented as $FeSO_4$, but damage of intestinal epithelium was observed suggesting increased permeability of the calves' duodenum to foreign pathogens and unregulated absorption of minerals together with other dietary components. However, their finding corresponds to ours on $Soil_{post}$ group. That hints to the significance of the kind of Fe compound reaching the duodenum. Antagonistic interactions between Fe, zinc, copper and manganese because of their competition for DMT1 as described by e.g., Davidson et al. (2015) and Suttle (2010) were not observed in the analysed organs, which is in contrast to findings in sheep (Grün et al., 1978) and goats (Schonewille et al., 1995). On the contrary, zinc and manganese contents were significantly increased in the ham of $Soil_{pre}$ group. In our study, the possible antagonism was probably compensated by a simultaneously higher supply of zinc and manganese through the added soil. The significantly lower DM concentration of the intestinal tissue in $Soil_{pre}$ group is an interesting observation. Ponka et al. (2015) described irritation and tissue damage of the gastrointestinal tract as first step of Fe poisoning. It is not clear whether the difference in DM contents is indicative of tissue damage, nevertheless other indications of gastrointestinal damage could be the LWG in $Soil_{pre}$ group, which amounted almost half of the other two experimental groups, and low feed conversion ratio in this group. In $Soil_{pre}$ group LWG was also depressed by relatively low feed intake which was however statistically similar to the control. Due to the lack of analytical methods, it was not possible to perform a speciation of the inorganic or organic forms in the digesta. This would be of high interest for future studies, as ensiling might increase organically bound fractions, which might have higher solubility. This could be relevant for the biological effects of and for the potential antagonistic impact on other trace minerals. It can be speculated that the observed lower feed intake might also be a consequence of a disturbed intermediary metabolism and other trace elements.

Conclusions

In the ensiling trials it was shown that soil contamination simulating conditions in agricultural practice can easily lead to high Fe concentrations in resulting silages. In ensiled material, Fe is present in a more reactive form leading to an increased Fe solubility and higher concentration in the intestinal tissue of goats. Under Fe abundance, goats are able to regulate Fe absorption; however, Fe levels in storage organs also were significantly increased. The rise is not proportional to intake, nevertheless enhanced when Fe is offered in ensiled forage. Although no antagonisms with other trace elements were observed, live weight gain was reduced after feeding soil, which was ensiled with the forage. Ensiling caused increased solubility and thus absorption of Fe. It remains to be investigated how intestinal tissue can tolerate longer periods of overload of ferrous iron, and if so, challenged by pathogenic microorganisms under these conditions.

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