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Influence of high inorganic selenium and manganese diets for fattening pigs on oxidative stability and pork quality parameters

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Data are scarce regarding combined high Se and Mn supplementation in livestock diets, however, as Se and Mn are functionally related as cofactors of glutathione peroxidase (GPx) and Mn-superoxide dismutase (SOD), respectively, beneficial synergistic effects on oxidative stability of tissues may result. This experiment evaluated the effect of an oversupply of Se and Mn within European legal limits compared with recommendations on performance, oxidative stability of the organism and meat quality in a randomised complete block design. A total of 60 crossbred gilts were fed maize–barley–soya bean meal diets formulated in a 2 × 2 factorial approach with inorganic Se (0.2 v. *0.5 mg/kg Se dry matter (DM)) or inorganic Mn (20* v. *150 mg/kg Mn DM) from 31 to 116 kg BW. Se supplementation reduced feed intake, whereas high Mn diets impaired average daily gain (*$P < 0.05$*). Qualitative carcass characteristics were impaired by Se and Mn predominantly in the* semimembranosus *muscle. Activity of GPx in liver was increased by high Se diets (*$P < 0.05$*). Mn supplementation increased catalase (CAT) activity in liver, GPx in plasma and total antioxidative capacity (TAC) in muscle, whereas it decreased CAT activity in plasma (*$P < 0.05$*). Cu/Zn-SOD in muscle showed higher activity in high-Se-low-Mn diets but lower activity when both high Se and Mn were combined (Se × Mn* $P < 0.05$*). Mn supplementation increased Mn concentration in* longissimus thoracis et lumborum*, but simultaneously reduced Se concentration (*$P < 0.05$*). Upon retail display, Mn increased lipid oxidation more pronouncedly (higher thiobarbituric acid reactive substances;* $P < 0.05$*) than Se (*$P < 0.10$*). Despite some positive effects (Mn increased TAC, Se increased GPx, Se and Mn increased tenderness), no synergistic effects of high Se and Mn diets or an overall beneficial impact on meat quality, especially during storage, could be observed. Including the negative effects on performance, feeding Se and Mn up to the maximum legal level cannot be recommended.*

Keywords: performance, carcass characteristics, antioxidant enzymes, lipid oxidation, retail display

Implications

Oxidation is the major non-microbial cause of quality deterioration in meat and is therefore one of the biggest economic problems in meat industry. It compromises nutritional quality, limits shelf life, increases toxicity and decreases market value of meat and meat products. Animal nutrition can influence these factors of meat quality. Providing further understanding in the reaction of an oversupply of inorganic Se and Mn on animal tissue can help to reduce negative effects of oxidation products on human health.

Introduction

A prooxidative environment due to reactive oxygen species (ROS) can impact the organism and the quality of the food of animal origin (Halliwell *et al.*, 1995). Meat quality is directly affected due to, for example, discoloration, off flavour, poor shelf life or drip loss. When polyunsaturated fatty acids (PUFAs) are oxidised by ROS, fatty acid radicals, and subsequently peroxy radicals, hydroperoxides and various aldehydes with potentially cyto- and genotoxic traits may be formed (Falowo *et al.*, 2014).

Various enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT)) and non-enzymatic (e.g. vitamin E, C, glutathione, uric acid) systems exist in the body to cover these prooxidative stressors and therefore reduce the susceptibility to oxidative stress (Halliwell *et al.*, 1995; Falowo *et al.*, 2014). Likewise, trace elements like selenium may affect oxidative stability, however, with inconsistent results concerning the nature of its impact: Skřivan *et al.* (2012) found beneficial impact of inorganic and organic dietary Se sources on oxidative stability of meat. In contrast, organic Se

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was reported to yield better drip loss and meat lightness than inorganic Se (Mahan *et al.*, 1999). Also manganese proved beneficial, when fed in high concentrations of mainly organic source (Apple *et al.*, 2004 and 2005).

Various synergistic or antagonistic effects of minerals and trace elements in absorption, transport and tissue storage are occurring (Suttle, 2010). Despite different absorption mechanisms, Se and Mn may yet interfere in nutrient metabolism, deposition or their physiological role, as Se and Mn are functionally related as cofactors of the antioxidative enzymes GPx and mitochondrial Mn-SOD, respectively. GPx contains selenocysteine at the active site and is responsible for the reduction of organic hydroperoxides (ROOH) to corresponding hydroxides (ROH) (Lawrence and Burk, 1978). SOD catalyses the reaction of superoxide anion radical to H_2O_2 (Marklund and Marklund, 1974). Therefore, Se and Mn may also influence the antioxidative capacity in the organism in protecting cells against damages caused by ROS and hydro- or lipid peroxides.

Depending on the concentration, impact of antioxidant molecules may shift from antioxidative to prooxidative (Halliwell *et al.*, 1995): The role of transition metals (e.g. in enzymes or metalloproteins) and oxidising lipids are discussed as factors influencing oxidation (Stadtman and Levine, 2003). Supplementing feed with nutritional components such as trace elements is therefore a balancing act.

In the European Union, for most species and categories, maximum dietary concentrations of 0.5 mg Se and 150 mg Mn/kg feed have been defined (European Commission, 2003). Setting these maximum concentrations, parameters such as performance, health, environmental aspects as well as consumer safety are taken into account; however, meat quality is considered to a lesser extent.

To our knowledge, no study has dealt with the inclusion of high Se and Mn diets in livestock. We hypothesise that an oversupply of dietary Se or Mn improves the oxidative stability of organism and animal product, showing synergistic effects when combined in feed of fattening pigs. Comparing recommendation and EU upper-level supplementation of inorganic Se and Mn in a 2×2 factorial approach, the impact on performance and various meat quality parameters was investigated.

Material and methods

Animals and housing

The experiment was conducted at the Austrian Pig Testing Facility (OESPA, Lower Austria, Austria). Each pen offered a minimum of 1.2 m^2/animal on fully slatted concrete floor and was equipped with a nipple drinker and an automatic dry-feeding system allowing individual determination of feed intake. A total of 60 Austrian crossbred ((Large White $\times$ German Landrace) $\times$ Piétrain) gilts, with an average initial BW of 32.4 ± 0.3 kg were allotted to 12 pens (i.e. five animals per pen, three replicates) on the basis of weight and litter, applying a randomised complete block design. Animals were marked with ear tag and transponder and monitored on a daily basis.

Diets and feeding

In a 2×2 factorial approach, inorganic Se and Mn were fed in two concentration levels (0.2 *v.* 0.5 mg/kg Se dry matter (DM); 20 *v.* 150 mg/kg Mn DM) for the whole fattening period. Considering the calculated native concentrations of Se and Mn (Table 1), Na-selenite and MnO were supplemented via vitamin and trace element premix to reach the proposed dietary concentrations. Animals received a grower diet (Table 1, 13.4 MJ metabolisable energy (ME)/kg, 16.4% CP, 0.94% standardised ileal digestible (SID) lysine as-fed) from start of the experiment up to an

Table 1 *Diet composition for grower and finisher phase*

	Grower phase	Finisher phase
Ingredient		
Maize (%)	39.47	63.63
Wheat (%)	22.50	–
Barley (%)	15.00	15.00
Soya bean meal without hulls (%)	18.00	15.00
Wheat bran	–	2.00
Limestone (%)	1.20	1.00
Monocalcium-P (%)	0.87	0.72
Salt (%)	0.36	0.36
Soya bean oil	1.50	1.50
L-Lysine (%)	0.35	0.20
DL-Methionine (%)	0.07	–
L-Threonine (%)	0.13	0.05
L-Tryptophan (%)	0.03	0.02
Vitamin and trace element premix (%)[1]	0.52	0.52
Phytase (FTU/kg)[2]	500	500
Calculated value (as-fed)		
ME (MJ/kg)	13.4	13.5
CP (%)	16.4	14.3
SID lysine (%)	0.94	0.73
SID methionine (%)	0.30	0.22
SID threonine (%)	0.63	0.50
SID tryptophan (%)	0.19	0.15
Ca (%)	0.68	0.57
Digestible P (%)	0.32	0.29
Na (%)	0.15	0.15
Fe (mg/kg)	140.9	135.6
Vitamin E (mg/kg)	35.8	36.7
Basal Se (mg/kg)	0.12	0.12
Basal Mn (mg/kg)	19.3	14.8

ME = metabolisable energy; SID = standardised ileal digestible.
[1]Amount per kg as-fed: vitamin A 8000 I.E.; vitamin D_3 1500 I.E.; vitamin E (as all-rac-α-tocopheryl acetate) 20.00 mg; iron (as iron-(II)-sulfate, monohydrate) 62.00 mg; copper (as copper-(II)-sulfate, pentahydrate) 11.00 mg; zinc (as zinc sulfate, monohydrate) 71.00 mg; iodine (as calcium iodate, anhydrous) 1.30 mg; cobalt (as alkaline cobalt-(II)-carbonate, monohydrate) 0.30 mg, diatomaceous earth as carrier; sodium selenite and manganese (II) oxide were added to the premixes to achieve the appropriate treatment levels.
[2]Unit of phytase activity (FTU); 6-Phytase from *Schizosaccharomyces pombe* (Phyzyme XP; Danisco Animal Nutrition, Marlborough, Wilts, UK).

average penwise BW of 60 kg. Subsequently, animals were fed a finisher diet (Table 1, 13.5 MJ ME/kg, 14.3% CP, 0.73% SID lysine as-fed). Isocaloric and isonitrogenous diets were based on maize, barley and soya bean meal and were provided in ground form. The experimental diets are formulated to meet or exceed the nutrient recommendations (Society of Nutrition Physiology, 2006). Animals were fed and watered *ad libitum*.

Slaughter, carcass characteristics and sampling

With an individual BW of about 115 kg, pigs were fasted for 12 h and slaughtered via stunning and bleeding at the adjacent slaughter facility. Blood samples were collected in heparinised tubes (Vacuette® Lithium Heparin; Greiner Bio-One, Kremsmuenster, Austria) during exsanguination. Following centrifugation (1500 × **g**, 4 min, room temperature), plasma was obtained and stored at −70°C. Tissue samples of liver (left lobe) and mere abdominal muscle (*rectus abdominis*) were collected, vacuum packaged, transported on crushed ice to the laboratory (~1 h) and stored at −70°C until analyses.

Carcass characteristics were determined at OESPA by skilled personnel. Qualitative parameters were analysed in *longissimus thoracis et lumborum* (LTL) and *semimembranosus* (SM) muscle. Loin (between the 13th and 14th thoracic vertebrae) and SM pH at 45 min *postmortem* was determined using a portable temperature compensating pH meter (Portamess; Knick, Berlin, Germany). Conductivity (Konduktometer L 191; WTW GmbH, Weilheim, Germany) was measured in LTL (between the 12th and 13th thoracic vertebrae at 45 min *postmortem* and between the 15th and 16th vertebrae at 24 h *postmortem*, each at a depth of 6 cm) and in SM (depth of 3 cm at both points in time). Following separation of LTL between 13th and 14th vertebrae 24 h *postmortem*, meat lightness was immediately determined at the dorsal part of the dissected loin area using an optometer (OPTO-Star; R. Matthäus, Nobitz/OT Klausa, Germany; Units of 'Göttinger Farbhelligkeitsmesser' <45 U = pale, soft, exudative (PSE); >80 U = dark, firm dry (DFD)). Drip loss – as the weight loss during a 24 h storage at 4°C on a grid – was determined in 50 g of a cubically cut loin sample obtained 24 h *postmortem*.

Boneless tissue samples of LTL were obtained 24 h *postmortem* from chilled carcass.

Analyses

Antioxidant enzyme activity. Liver and abdominal muscle were homogenised in a potassium phosphate buffer (50 mmol/l K_2HPO_4, 50 mmol/l KH_2PO_4, 1 mmol/l ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100) using an Ultra Turrax T25 homogeniser (IKA® Werke GmbH und Co. KG, Staufen, Germany). The buffer for GPx activity determination additionally contained 1 mmol/l of NaN_3. Activities of GPx (Lawrence and Burk, 1978), total SOD as well as Mn-dependent SOD and Cu/Zn-SOD (Marklund and Marklund, 1974) and CAT (Beers and Sizer, 1952) were determined. Activities in plasma were determined without prior homogenisation.

Chemical analyses. Analyses of DM, CP, ether extracts (EE) and ash in feed and LTL as well as crude fibre, starch and sugar additionally in feed were performed (Naumann and Bassler, 2012). Minerals and trace elements were determined using inductively coupled plasma mass spectrometry (ICP-MS) analysis after wet digestion with HNO_3 and H_2O_2. Measurements were performed on a double-focussing sector field ICP-MS (Finnigan ELEMENT2; Thermo Electron Corporation, Bremen, Germany) equipped with a CETAC ASX-520 autosampler (CETAC Technologies, Omaha, NE, USA), a cyclonic spray chamber (20 ml, Jacketed Cinnabar Cyclonic, Glass Expansion, West Melbourne, Australia) and a conical nebuliser made from borosilicate glass (Glass Expansion). Argon (Ar 4.6, 99.996%) cool gas flow was 16 l/min, auxiliary (plasma) gas and sample (nebuliser) gas flows were optimised daily to obtain maximum signal intensity, the former typically between 0.75 and 0.90 l/min, the latter in the range of 0.90 and 1.00 l/min. Radio-frequency (RF) power was 1190 W. Thiobarbituric acid reactive substances (TBARS) were determined in liver, abdominal muscle and in LTL (before and after retail display, see below). Tissue homogenates (Ultra Turrax T25) with 1.15% ice cold KCl buffer including 0.3% butylated hydroxytoluene were produced and TBARS were determined according to Ohkawa *et al.* (1979) using 5 ml of butanol for transfer of reaction products to the organic phase. Values are expressed as mg malondialdehyde equivalents per kg of wet tissue. Total antioxidative capacity (TAC) was analysed using Cuprac method (Apak *et al.*, 2007) and is presented as ascorbic acid equivalents.

Sensory aspects and retail display of longissimus thoracis et lumborum *chops*. A sensory panel of six persons experienced in sensory evaluation of meat was used. Samples were thawed to 2°C for 24 h before testing. Untreated 1 cm thick LTL chops were cooked well-done for 5 min on each side in individual dishes at maximum power (Raclette Gourmet Grill Deluxe, Tefal S.A.S., Rumilly, France) and served immediately. Water and white bread were provided for neutralisation of receptors. Each panellist evaluated four pork samples per session (i.e. 15 sessions in total, performed on 2 days), one sample from each treatment in randomised order, for tenderness, juiciness and flavour intensity, using a six-point hedonic scale, in which 1 = very tough/very dry/low flavour intensity and 6 = very tender/very juicy/high flavour intensity. Sample of each session were additionally ranked in order of subjective overall preference (lower value = higher preference).

Starting 24 h *postmortem*, LTL chops were subject to a 7-day retail display simulation. Chops were plotted dry, weighed, vacuum packed and stored under continuous light (500 to 800 lx, Basics T5 fresh colour fluorescent tube, 14 W; Bäro, Leichlingen, Germany) in a cooler (4°C). Weight loss during storage (%) was determined gravimetrically on the patted dry loin chop. pH value was analysed in duplicate using a calibrated temperature compensating pH meter (testo 206 pH2; Testo AG, Lenzkirch, Germany) before (i.e. 24 h *postmortem*) and after retail display.

Statistical analysis. Data were evaluated with two-way ANOVA (PROC MIXED, SAS 9.4; SAS Institute, Cary, NC, USA) including the experimental factors (Se, Mn), their interaction (Se × Mn) and the replicates in the model. Animal or the tissue derived thereof served as experimental unit. Weekly data were used to evaluate performance (average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR)) applying repeated measures. For retail display (TBARS, pH) repeated measures of display days were accounted for. Panellist served as random effect in sensory evaluation. Pearson's correlation coefficient was determined applying PROC CORR. Results are presented as least square means and pooled standard error of means.

Results

Feed, performance and carcass characteristics

Results of feed analyses and performance are presented in Tables 2 and 3. In grower feed Se supplementation was not observable despite the verification of supplementation in the premix (inhomogeneity of feed). No severe morbidities or mortalities of pigs were recorded. High Se concentration in diets reduced ADFI in all growth periods investigated, without concomitantly improving FCR. High Mn supplementation resulted in reduced ADG in the finisher period and the overall performance. In finisher phase, ADFI was significantly lower in all supplemented groups compared with the control (Se × Mn $P < 0.05$), however, additive effects of element combination were not found.

Quantitative carcass characteristics (Table 4) were only insignificantly influenced by Se or Mn supplementation. High Mn diets showed a trend in decreasing LTL fat area ($P < 0.10$). Changes in qualitative carcass characteristics were predominantly observed in SM muscle, showing a significant interaction for pH (45 min *postmortem*), with reductions in single high supplementation. Conductivity in SM was increased by Mn 45 min *postmortem* ($P < 0.10$), whereas single high doses of Se increased conductivity after 24 h in SM but not in LTL. Drip loss and lightness remained uninfluenced by dietary intervention.

Oxidative properties

Activity of GPx in liver was increased due to high Se diets (Table 5). Furthermore, Mn increased liver CAT activity, whereas activity of SODs remained unchanged. Changes in enzyme activity were not reflected in changes in the concentration of TBARS or TAC. In abdominal muscle, Cu/Zn-SOD was increased in activity by the Se-high diet (Se × Mn $P < 0.05$). Furthermore, dietary Mn supplementation increased muscle TAC. In plasma, Mn increased GPx activity, while reducing CAT.

Nutrient composition and organoleptic properties of LTL

High dietary Se and Mn concentrations had no influence on DM, CP and EE in LTL samples (Table 6). Nevertheless, there was a lower ash concentration when high amounts of trace elements were applied; however, without additive effects when factors were combined (Se × Mn $P < 0.10$). In detail, these changes can be traced back to decreased potassium concentrations due to Mn supplementation ($P < 0.10$) and reduced phosphorus concentrations in single high Se/Mn diets ($P < 0.10$). The Se increasing effect of Se supplementation was only visible when calculation was performed in μg/kg FM ($P < 0.10$; data not shown) but not in DM. High dietary Mn could increase Mn in LTL; however, Mn markedly decreased Se concentration ($P < 0.05$). Results of organoleptic analyses (Table 7) showed a higher intensity of flavour along with lower juiciness with Se supplementation ($P < 0.10$) and higher tenderness in single high trace element diets (Se × Mn $P < 0.01$).

Retail display

There was a trend of higher TBARS concentrations and reduced pH value in the non-stored (= 24 h *postmortem*) LTL of the Mn group (Table 8). After retail display no statistically determinable difference between treatments was observable concerning TBARS, ultimate pH value or weight loss. Nevertheless, the overall effect of retail display on TBARS concentration (repeated measures) showed an increasing effect of Mn ($P = 0.015$) as well as a trend of Se ($P = 0.091$) to increase pork TBARS.

Table 2 *Analysed nutrient composition in experimental diets (as-fed)*

	Grower phase				Finisher phase			
	Control	Se high	Mn high	Se + Mn high	Control	Se high	Mn high	Se + Mn high
Dry matter (g/kg)	893	892	891	890	886	887	888	891
ME (MJ/kg)[1]	14.0	14.1	14.1	13.9	13.9	14.0	14.0	14.0
CP (g/kg)	161	165	164	162	144	142	139	140
Ether extracts (g/kg)	31.5	33.7	35.2	32.1	36.4	41.2	45.0	42.2
Crude fibre (g/kg)	22.9	22.6	23.9	24.3	27.4	25.4	26.9	25.7
Ash (g/kg)	48.8	47.8	45.8	51.2	43.8	42.1	44.0	46.1
Starch (g/kg)	482	484	477	488	498	508	501	508
Sugar (g/kg)	39.6	40.4	39.8	40.0	37.1	34.9	34.5	35.2
Se (mg/kg)	0.37	0.37	0.38	0.37	0.37	0.52	0.30	0.47
Mn (mg/kg)	38.40	44.21	110.49	148.34	40.5	33.0	115.6	152.3

[1]Metabolisable energy (ME) calculated from data provided by Society of Nutrition Physiology (2008).

Table 3 *Performance results of fattening pigs fed an oversupply of Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
Fattening duration (days)	109.7	112.5	111.7	116.7	1.6	0.237	0.342	0.739
Starting BW (kg)	32.7	32.2	32.1	32.4	0.3	0.746	0.637	0.364
Final live BW (kg)	116.6	117.1	115.9	116.7	0.2	0.171	0.260	0.808
Average daily gain (g/day)								
32 to 60 kg	763	755	741	730	9	0.613	0.188	0.932
60 to 116 kg	778	775	747	725	9	0.477	0.033	0.610
Overall	772	766	744	720	7	0.265	0.006	0.482
Average daily feed intake (g/day)								
32 to 60 kg	1712	1653	1702	1556	19	0.010	0.166	0.262
60 to 116 kg	2313[a]	2130[b]	2191[b]	2148[b]	17	0.001	0.097	0.026
Overall	2074	1930	1989	1929	15	0.001	0.158	0.166
Feed conversion ratio (kg/kg)								
32 to 60 kg	2.25	2.19	2.26	2.19	0.02	0.141	0.894	0.953
60 to 116 kg	2.95	2.80	2.97	2.97	0.03	0.202	0.123	0.185
Overall	2.69	2.54	2.64	2.65	0.02	0.110	0.527	0.104

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4 *Carcass characteristics of fattening pigs fed an oversupply of Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
Hot carcass weight (kg)	93.2	94.0	92.9	93.0	0.3	0.386	0.213	0.542
Dressing (%)	80.0	80.3	80.3	79.7	0.2	0.671	0.783	0.206
Lean (%)[1]	60.2	60.0	59.8	60.5	0.2	0.661	0.921	0.417
Valuable cuts (%)	51.5	51.0	51.3	51.5	0.2	0.683	0.506	0.321
Fat : meat[2] = 1 : *x*	7.91	7.60	8.10	8.17	0.18	0.738	0.296	0.597
Body length (cm)	102.4	102.7	102.7	103.3	0.4	0.614	0.447	0.867
Backfat depth (mm)[3]	19.1	20.1	18.9	18.8	0.3	0.445	0.179	0.378
Loin depth (mm)	73.1	74.0	71.8	73.6	0.5	0.220	0.412	0.679
LTL area (cm^2)	56.7	55.2	55.6	56.2	0.5	0.677	0.986	0.364
LTL fat area (cm^2)	15.4	15.1	14.9	13.3	0.3	0.158	0.078	0.331
pH 45 min, LTL	6.18	6.17	6.21	6.12	0.04	0.534	0.910	0.567
pH 45 min, SM[4]	6.43	6.19	6.14	6.28	0.04	0.550	0.252	0.035
Conductivity, 1 h, LTL (mS/cm)	3.93	3.88	4.08	4.03	0.05	0.643	0.154	0.987
Conductivity, 1 h, SM (mS/cm)	4.39	4.27	4.77	4.45	0.08	0.174	0.085	0.534
Conductivity, 24 h, LTL (mS/cm)	4.41	4.63	4.68	5.26	0.27	0.476	0.426	0.754
Conductivity, 24 h, SM (mS/cm)	4.21[b]	7.95[a]	6.95[ab]	6.60[ab]	0.46	0.059	0.436	0.024
LTL lightness (U[5])	64.3	64.1	65.1	64.6	0.5	0.699	0.446	0.859
Drip loss, LTL (%)	2.97	3.32	3.60	4.14	0.26	0.401	0.170	0.856

LTL = *longissimus thoracis et lumborum*; SM = *semimembranosus* muscle.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Lean % = 48.7719 − 0.48330 × backfat depth (thinnest backfat section above *M. gluteus medius*) +0.23127 × loin depth (BMLF, 2011).
[2](Weight of loin + filet + ham)/(weight of fat overlay of loin + ham).
[3]Mean of three measurements: thickest section over the first thoracic vertebra + thinnest section over the last thoracic vertebra + thinnest section above *M. gluteus medius.*
[4]$P > 0.10$ for *post-hoc* test (Tukey).
[5]Units of 'Göttinger Farbhelligkeitsmesser' <45 U = PSE; >80 U = DFD (OPTO-Star).

Discussion

Performance and carcass characteristics

As dietary Se concentration is reported to have no impact on performance at recommendation level (Society of Nutrition Physiology, 2006) and only starts impairing at 5 mg/kg Se (Kim and Mahan, 2001) no changes were expected. Nevertheless, Se reduced ADFI without showing improved FCR. Likewise, also Mn is reported to be safe up to dietary concentrations of 1000 mg/kg (National Research Council, 2005). Despite that,

Table 5 *Enzyme activity, thiobarbituric acid reactive substances (TBARS) and total antioxidative capacity (TAC) (Cuprac method) in liver and muscle (*rectus abdominis*) homogenates and plasma of fattening pigs fed an oversupply of Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
Liver homogenates								
GPx (U/g protein)	204.3	234.8	215.3	220.7	4.6	0.049	0.870	0.162
SOD (U/mg protein)	11.96	12.93	12.23	12.75	0.37	0.332	0.952	0.766
Mn-SOD (U/mg protein)	2.67	2.56	2.63	2.65	0.08	0.723	0.948	0.624
Cu/Zn-SOD (U/mg protein)	9.27	10.37	9.61	10.14	0.34	0.248	0.959	0.657
CAT (U/mg protein)	892.2	912.6	986.3	976.6	16.2	0.865	0.014	0.633
TBARS (mg/kg)	142.6	142.8	142.7	140.6	1.5	0.759	0.724	0.705
TAC Cuprac[1]	10.51	10.59	10.73	10.81	0.08	0.583	0.176	1.000
Muscle homogenates								
GPx (U/g protein)	12.46	12.77	10.91	13.31	0.42	0.107	0.546	0.212
SOD (U/mg protein)	1.33	1.34	1.23	1.27	0.03	0.652	0.163	0.816
Mn-SOD (U/mg protein)	1.03	0.95	0.92	0.97	0.03	0.817	0.338	0.245
Cu/Zn-SOD (U/mg protein)	0.30[ab]	0.39[a]	0.35[ab]	0.26[b]	0.02	0.927	0.233	0.007
CAT (U/mg protein)	6.37	6.28	6.14	6.27	0.12	0.934	0.644	0.678
TBARS (mg/kg)	18.99	20.34	20.00	20.19	0.45	0.410	0.646	0.533
TAC Cuprac[1]	3.95	4.02	4.11	4.10	0.03	0.602	0.037	0.500
Plasma								
GPx (U/ml)	1.03	0.98	1.13	1.16	0.03	0.849	0.017	0.438
CAT (U/ml)	190.1	195.4	152.7	148.8	4.9	0.932	0.0001	0.587

GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]mg ascorbic acid equivalents/g wet tissue.

Table 6 *Nutrient content in* longissimus thoracis et lumborum *of fattening pigs fed an oversupply of inorganic Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
Crude nutrients								
DM (%)	25.64	25.90	25.95	25.96	0.06	0.218	0.104	0.259
Ash (% DM)	4.55	4.40	4.41	4.41	0.02	0.078	0.094	0.067
CP (% DM)	92.32	90.70	91.02	90.73	0.34	0.161	0.351	0.328
EE (% DM)	5.60	5.61	5.76	6.20	0.21	0.604	0.382	0.623
Minerals and trace elements (/kg DM)								
Mg (mg)	1044	1029	1021	1028	5	0.657	0.194	0.226
Na (mg)	1639	1663	1655	1655	15	0.690	0.903	0.708
K (g)	16.45	16.15	16.03	15.95	0.08	0.296	0.092	0.526
P (g)	6.88	6.81	6.79	6.91	0.02	0.667	0.983	0.063
S (g)	7.10	7.07	7.02	7.05	0.02	0.977	0.367	0.515
Fe (mg)	15.02	14.97	15.14	15.32	0.19	0.864	0.545	0.761
Zn (mg)	37.64	38.26	36.86	37.07	0.47	0.665	0.306	0.830
Cu (mg)	1.41	1.43	1.35	1.38	0.02	0.529	0.245	0.904
Se (μg)	294.9	309.6	272.7	288.7	5.1	0.130	0.035	0.948
Mn (μg)	209.8	214.9	225.7	232.2	3.4	0.381	0.015	0.922

DM = dry matter; EE = ether extracts.

we observed impaired ADG due to high Mn supplementation especially in the older animals. High inorganic Mn in feed in a hot and humid environment may lead to peroxidation (Suttle, 2010) or altered intestinal absorption (Smith *et al*., 1995). Thus, the performance impairing effect observed may in part have been indirectly caused by reduced feed quality due to hot outdoor temperature.

No influence of Se supplementation on quantitative carcass characteristics was recorded. Manganese, however, decreased LTL fat area ($P < 0.10$). Increasing dietary Mn

Table 7 *Results of organoleptic analyses of* longissimus thoracis et lumborum *obtained from fattening pigs fed an oversupply of Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
Tenderness[1]	2.83[b]	3.11[ab]	3.24[a]	2.81[b]	0.04	0.377	0.549	<0.001
Juiciness[1]	3.08	2.88	3.14	3.01	0.04	0.053	0.264	0.714
Flavour[1]	3.08	3.28	3.12	3.21	0.04	0.056	0.884	0.488
Overall preference[2]	2.44	2.57	2.43	2.57	0.05	0.196	0.957	0.957

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Six-point hedonic scale, in which 1 = very tough/very dry/low flavour intensity and 6 = very tender/very juicy/high flavour intensity.
[2]Ranked in order of subjective preference (lower value = higher preference).

Table 8 *Thiobarbituric acid reactive substances (TBARS) content, pH value and weight loss (%) during 7-day retail display (day 0 = 24 h* postmortem*) of* longissimus thoracis et lumborum *obtained from fattening pigs fed an oversupply of Se and Mn compared with recommendations*

	Treatment					*P*-value		
	Control	Se high	Mn high	Se + Mn high	SEM	Se	Mn	Se × Mn
Animals (*n*)	15	15	15	15				
TBARS, day 0 (mg/kg)	1.59	1.63	1.68	1.81	0.04	0.234	0.053	0.519
TBARS, day 7 (mg/kg)	1.74	1.82	1.83	1.99	0.04	0.163	0.120	0.640
pH, day 0	5.38	5.45	5.37	5.37	0.01	0.112	0.061	0.109
pH, day 7	5.38	5.38	5.37	5.37	0.01	0.797	0.590	0.994
Weight loss (%)	5.32	5.21	5.20	5.83	0.13	0.347	0.357	0.171

concentrations also led to quadratically reduced backfat depth (Apple *et al.*, 2004), which may be traced back to lipoprotein lipase activity in fat tissue (Lu *et al.*, 2006).

We found decreased pH in SM muscle due to single high supplementation of Se or Mn. Similarly, also the conductivity in SM was increased, indicating a trend towards pale, soft and exudative meat.

Interestingly, feeding intervention did not show comparable effects in the different muscles analysed. More pronounced changes were observed in SM than in LTL muscle. Supplementing inorganic Se had a stronger effect on Se concentration in SM than LTL (Lisiak *et al.*, 2014). Higher TBARS concentration after storage (Bobček *et al.*, 2004) and higher concentrations of PUFAs, as well as Fe, Zn, Cu (Purchas *et al.*, 2009) can be found in SM compared with LTL, so SM seems more susceptible to changes in dietary trace element concentrations.

Oxidative properties

In finishing pigs, serum GPx activity is reported to reach a plateau at a dietary level of 0.1 mg/kg Se as Na-selenite (Mahan *et al.*, 1999), thus explaining our lack of improvement in plasma. GPx activity in liver and muscle can be increased when supplementing Se to a Se-deficient control (Zhan *et al.*, 2007). Interestingly, we found higher GPx activity in liver raising inorganic Se from recommendation to maximum dietary EU level, indicating, that no plateau was yet reached in liver. Alternatively, higher GPx activity may have become necessary due to the prooxidative potential of selenite, as reviewed by Mézes and Balogh (2009) and discussed below. Different reaction patterns of Se supplementations in the tested matrices (liver, muscle, plasma) may be a result of different GPx concentration and activity levels (Daun and Åkesson, 2004) and therefore different Se concentrations in the physiological form.

Single high supplementation of Se resulted in an increased Cu/Zn-SOD activity in abdominal muscle. Higher SOD activity due to Se was also observed by Ahmad *et al.* (2012), who proposed an influence of several selenoproteins on thyroid function, provoking the production of ROS. Interestingly, combined Mn and Se supplementation alleviated this effect, resulting in an interaction of the factors ($P < 0.05$).

Supplemental Mn did neither alter Mn-SOD nor Cu/Zn-SOD or the total activity, indicating sufficient Mn concentrations in control diets to reach a saturation state. Dietary Mn increased the activity of CAT in the liver but decreased it in plasma. Even though a procaryote CAT is reported to contain Mn in the catalytic active site, CAT in eukaryotes carries Fe in a haem group (Whittaker, 2012), thus suggesting a relation of Mn with Fe in the current study. Mn and Fe are also interrelated in the eukaryote organism, using the same absorption mechanism and transport (Suttle, 2010). This may directly or indirectly also result in an interaction with CAT activity. Yet the question remains why the effect of high Mn on CAT activity was opposed in the two matrices. In general, the relation of total Fe : Mn decreases from plasma > muscle > liver (Suttle, 2010) and therefore may not be responsible for the observed effect. Zheng *et al.* (1999) reported an upregulation of cellular Fe uptake in chronic

Mn exposure. This resulted in reduced Fe concentrations in plasma while transferrin remained unchanged. Even though we have not analysed absolute Fe concentrations in liver and plasma, we hypothesise that lower available Fe in plasma and higher concentrations in liver may have affected these opposed changes in CAT activity.

CAT and GPx can both use H_2O_2 as a substrate. Therefore, the increase in GPx activity in plasma seen in Mn-supplemented pigs may be a compensational reaction to the decrease in plasma CAT activity ($r = -0.273$, $P = 0.0459$).

Higher GPx activity in liver or Cu/Zn-SOD in muscle should go in line with a decrease in TBARS concentration, as marker of lipid peroxidation, resulting in improved oxidative stability (Lu *et al.*, 2006; Zhan *et al.*, 2007). However, there is no correlation neither in our study (GPx *v.* TBARS: $r = 0.042$, $P = 0.747$; Cu/Zn-SOD *v.* TBARS: $r = 0.248$, $P = 0.056$) nor in the trial of Ahmad *et al.* (2012). Determination of TBARS may therefore be no sensitively enough method to record these changes.

Aside from antioxidative enzymes, low molecular weight antioxidants of dietary or endogenous origin are also responsible for oxidative stability. In this respect, Se is reported to interact with dietary antioxidants such as vitamin E (Suttle, 2010), thus, a relation of Se with the antioxidative capacity could be expected (Ahmad *et al.*, 2012), but was not observed. In contrast, Mn increased antioxidative capacity in abdominal muscle, this, however, was not reflected in lower TBARS concentrations.

Nutrient composition and organoleptic properties of LTL

Feeding high Se and Mn diets hardly affected nutrient concentrations of LTL, despite a trend in lowering ash concentrations ($P < 0.10$). Lower ash concentration of breast meat was also found by Ahmad *et al.* (2012) supplementing Na-selenite to broiler diets.

Se concentration in LTL was not as markedly elevated due to Na-selenite supplementation as can be seen when Se-deficient diets serve as control (Zhan *et al.*, 2007). Livestock does not accumulate high dietary Mn concentrations (National Research Council, 2005) as absorption and elimination via bile/faeces are well regulated. Despite that, we could see increases in tissue Mn concentration by 7.8%.

Even though Mn is functionally related with Mg (Suttle, 2010), no influence of Mn supplementation on the Mg concentration in LTL was observed. Nevertheless, there was a correlation between Mn and Fe concentration in LTL ($r = 0.503$; $P < 0.0001$), supporting the relation discussed earlier. Interestingly, our results indicate that high dietary Mn has a reducing effect on Se tissue concentration. So despite different absorption and transport mechanisms there may be an effect on tissue element composition. Mn-SOD or non-proteinaceous Mn antioxidants may have taken over the antioxidative protection (Aguirre and Culotta, 2012), so we hypothesise that not only GPx but also other selenoproteins (e.g. selenoprotein W) may have been required to a lesser extent. Whether Mn indeed affects functional selenoproteins, Se homoeostasis (e.g. by increasing renal excretion) or tissue storage remains to be elucidated.

In general, higher oxidation is related with a lower tenderness sensation due to oxidation and crosslinking of proteins (Lund *et al.*, 2007). This suggests that the increase in tenderness in both single high applications of Se or Mn may be a result of lower predisposition to oxidation. However, there was no recordable correlation ($r = -0.15$; $P = 0.243$) of tenderness with TBARS, and lipid oxidation in general was increased with high trace element supplementation.

Retail display

Dietary Se or Mn can improve stability of meat, for example, during retail display (Apple *et al.*, 2005; Zhan *et al.*, 2007). This can be observed in reduced lipid peroxidation, weight loss or improved colour stability. Contradicting these results, we found increased TBARS concentration in retail display of LTL when MnO ($P < 0.05$) and Na-selenite ($P < 0.10$) were fed. Prooxidative effects of very high Mn sulphate concentrations in an *in vitro* model were reported (Tjhio and Karel, 1969). Most experimental studies supplementing Mn in livestock diets used amino acid chelates or Mn sulphate as sources. The authors are not aware of data available on the effect of MnO on storage stability and lipid oxidation in meat. In contrast, reports are available on the potentially detrimental effects of inorganic Se sources such as selenite (Mahan *et al.*, 1999). The oxidation of thiols like glutathione by selenite and other Se compounds can produce superoxide or H_2O_2 (Spallholz, 1994). So even though we did not see negative changes of SOD or TBARS in liver and the non-stored abdominal muscle, prooxidative traits of inorganic Se might only be brought about in a prooxidative environment such as during retail display.

Conclusions

Feeding inorganic Se above recommendation levels could still increase liver GPx activity. While no further activity increase of Mn-SOD was observed, high Mn diets showed interactions with Fe-dependent antioxidative defence. Although only numerical increases in Se tissue concentration following Se supplementation were recorded, concomitant increase of Mn even had a Se-lowering effect. Despite some positive effects (TAC increasing effect of Mn, GPx increasing effect of Se), no synergistic effects of high Se and Mn diets could be observed. Interactions of factors showed attenuated impact of a combined supplementation compared with single high doses, yet the control supplemented with recommended concentrations offered the most favourable values. Feeding fattening pigs with concentrations as high as the EU maximum levels may lead to adverse effects on meat quality. Given the performance impairing effect, the question arises if dietary maximum levels for fattening pigs should be reconsidered.

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