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Beneficial antioxidant status of piglets from sows fed selenomethionine compared with piglets from sows fed sodium selenite

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ABSTRACT

Background: Studies in mammals proved dietary organic selenium (Se) being superior to inorganic Se regarding effects on growth performance, antioxidative status, immune response, and Se homeostasis. However, the picture of possible effects of different Se sources and – levels can be expanded. The present field study evaluated the effects on weight gain, hematological and selected biochemical variables as well as plasma concentrations of vitamin E (vitE), total Se and selenobiomolecules in piglets throughout the suckling period.

Methods: Piglets were monitored from birth to 38 days of age (d). The mother sows' diets were enriched with L-selenomethionine (SeMet-0.26 and -0.43 mg Se/kg feed) or sodium selenite (NaSe-0.40 and -0.60 mg Se/kg feed) from 1 month prior to farrowing until the end of lactation period. Piglets received pelleted feed supplemented with Se similarly to the sows' diets from one week of age. Selenite at 0.40 mg Se/kg (NaSe-0.40) represents a common Se source and -level in pig feed and served as control diet.

Results: From 24d, piglets in SeMet-groups had higher mean body weight (BW) compared with piglets from sows fed NaSe-0.40. Furthermore, from five-d and above, piglets from sows fed NaSe-0.60 had significantly higher BW than offspring from sows fed NaSe-0.40. Neonatal piglets in group SeMet-0.43 had significantly lower red blood cell counts (RBC), hemoglobin (Hgb) and hematocrit (Hct) concentrations compared with piglets from sows fed with NaSe-0.40. Neonatal and 5d-old piglets in group SeMet-0.26 showed higher gamma-glutamyl transferase activity than piglets in group NaSe-0.40. From five d and above, group NaSe-0.60 excelled with increased specific hematological variables culminating at age 38d with increased Hct, mean corpuscular volume (MCV), and MC hemoglobin (MCH) as well as increased activities of aspartate transaminase and lactate dehydrogenase compared with the other groups. Generally, offspring in the SeMet groups had higher total Se-concentrations in plasma than those from sows fed selenite, and showed a dose-response effect on plasma Se-concentrations. Furthermore, SeMet-fed piglets had higher plasma levels of the selenoproteins (Sel) glutathione peroxidase 3 (GPx3) and SelP as well as selenoalbumin. Plasma vitE levels were significantly negatively correlated with RBC throughout trial period.

Conclusions: Maternal supplementation with SeMet during gestation influenced hematology and clinical biochemistry in neonatal piglets in a different way than in offspring from sows receiving selenite enriched diets. Growth performance was positively influenced by both dietary Se source and Se level. Higher plasma levels of GPx3 observed in piglets receiving SeMet probably improved the protection against birth or growth related oxidative stress. These might prime the piglets for demanding situations as indicated by higher weight gain in offspring from sows fed with SeMet-supplemented diets. Our results on some enzyme activities might indicate that piglets fed NaSe-0.60 had to cope with increased levels of oxidative stress compared with those originating from sows fed SeMet or lower dietary levels of selenite. We assume that combining inorganic and organic Se sources in complete feed for breeding sows might be beneficial fro reproduction and the offspring's performance.

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1. Introduction

Goals in modern pig breeding unite high daily weight gain, mainly based on rapid muscle growth, with high feed efficiency [1]. Modern sows are the result of targeted selection for litter size and weight in maternal lines, which has led to a substantial increase in the sows' milking ability and improvement of maternal traits [2,3]. As an example, Norwegian piglets are 700 g larger at 21 days of age on average in 2012 compared with 2004 [2]. However, the high growth rate is associated with enhanced levels of reactive oxygen species (ROS) [4,5] and is possibly linked to a higher prevalence of selenium (Se) deficiency in pigs [6]. A delicate redox balance must exist to allow for proper growth and development [7]. A beneficial antioxidant status in sows could attenuate oxidative stress-related long-lasting effects on the offspring [8,9]. Most of the selenoproteins (Sel) are oxidoreductases and several act as antioxidants [10–14]. As the decisive structural component of Sel, Se is an essential micronutrient [15,16]. Prenatal Se supplementation through the dam can provide an effective antioxidant status at birth while postnatal Se-supplementation is the main determinant of progeny Se status after the first days postpartum [17]. However, recent reports indicate that supplementation with sodium selenite already from levels of 0.3 mg/kg on increase the oxidative stress levels in modern, fast-growing, feed efficient pigs [18–20]. Metabolizing the rather strong oxidizing selenite can trigger endoplasmic reticulum stress due to the generation of ROS [21,22]. Furthermore, Hu et al. [23] suggested that maternal intake of L-selenomethionine (SeMet) might increase the sows' productive performance due to increased transfer of Se to its offspring compared with maternal intake of selenite. We could corroborate an increased transfer of Se from sows to their offspring when fed SeMet [19]. Thus, proper feeding strategies applied to sows are essential as there might be carry-over effects contributing to the optimization of herd profitability by achieving more uniform litter weights and improving piglet performance [24].

Vitamin E (vitE), a crucial chain-breaking and lipid-soluble antioxidant in blood, plasma and cell membranes [24–26], is another vital nutrient for growth and health status of pigs [25]. The primary biological function of vitE, as well as of Se in the Sel glutathione peroxidase (GPx), is to prevent oxidative damage of cell membranes by converting ROS [26, reviewed in 27] into non-reactive forms [28]. ROS are usually generated during cellular respiration or respiratory burst [29,30]. Furthermore, vitE and Se status are known to influence immunological functions and disease resistance in pigs [31,32].

In previous studies on grower-finisher pigs and sows, we showed that inorganic and organic Se-sources differently influenced clinical biochemical variables as e.g. glutamate dehydrogenase (GLDH) activity, plasma levels of some minerals including Se, and selenobiomolecules (Se biomolecules) in plasma, colostrum, and milk, and the feed intake of lactating sows [18,19]. The sows giving birth to the offspring included in this study and later on their offspring were fed with diets supplemented with the Se sources L-selenomethionine (SeMet) or selenite (´NaSe´) at levels around and above the maximum allowed limit [19,33,34] according to the European Union legislation. The present trial aimed to determine if Se from these Se sources and supplemented each at two levels differently influences weight gain, hematological and clinical biochemical variables as well as plasma vitE, total Se and Se biomolecules in their offspring.

2. Materials and methods

2.1. Study design and animal ethics

The trial was approved by the Norwegian Food Safety Authority. It complied with the current EU and Norwegian Animal Welfare Act (LOV-2009-06-19-97 and LOV-2015-06-19-65, respectively) and the Norwegian regulations on swine husbandry (FOR-2003-02-18-175).

In this study on a commercial facility, a random selection of piglets from 31 sows was included. Parental genetics, maternal housing conditions and the feeding trial in the sows were described elsewhere [19]. In brief, the maternal feeding trial started one month prior to farrowing and lasted until the end of the lactation period. About one week before farrowing, the sows were moved into the farrowing unit consisting of individual farrowing pens. Within the farrowing pens (7.2 m^2), an area of 1.9 m^2 was slatted floor, and piglets' creep area with heating lamp comprised 1.1 m^2. At farrowing, the room temperature was set to 20 °C and reduced to 18 °C over ten days. Directly after birth, adhering fetal membranes were removed, and piglets were dried with paper and placed in the closed piglet creep area until weighing and blood sampling. Before sampling, the navel was disinfected with a 10% povidone-iodine solution. The first day all piglets received an oral treatment with iron (pulp with 180 mg Fe/mL as iron dextran and iron(II) chelate amino acid hydrate in soy oil; Pluss jernstarter Felleskjøpet, Norway). Furthermore, the litters received ca. 0.2 L of iron-enriched peat daily (Felleskjøpet Pluss Smågristorv, Norway). The piglets were introduced to pelleted feed one week after farrowing and *ad libitum* feeding started at weaning, i.e., at 33.6 ± 1.3 days of age. The composition of the pelleted feed and its nutritional values are listed in Table 1. Water was provided *ad libitum* throughout the study.

Male piglets were castrated under local anaesthesia (20 mg Lidocain + 18 µg adrenaline intratesticularly and subcutaneously in the scrotum) and systemic non-steroidal anti-inflammatory drugs (NSAID)

Table 1
Dietary components in basal diet and chemical composition for all diets (expressed as mg/kg feed, percentage of dry matter and mean concentrations (SD)).

Ingredient	Sows diets	Piglets/weaners diets
Barley	30.0	20.0
Soy beans	11.3	
Wheat	20.0	39.9
Wheat bran	7.0	
Oats	3.0	
Horsebeans	5.0	
Pea starch	1.7	8.5
Soy bean oil		0.7
Beet pellets	4.0	
Animal fat	2.2	3.2
Feed lime	1.6	1.1
Mono-calcium phosphate	0.56	0.8
Feed salt	0.34	0.2
Mikromin Svin	0.13	0.2
Vitamin A	0.07	0.1
Vitamin ADKB	0.07	
L-Lysine	0.24	0.7
DL-Methionine	0.03	0.3 (analogue)
L-Threonine	0.08	0.2
L-Tryptophan	0.19	0.1
Phyzyme XP 5000 TPT	0.014	0.01
Dry matter (%)	87.1	87.5
Water (% of DM)	12.9	12.5
Protein (% of DM)	15.4	18.8
Fat ((% of DM), hydrolysis)	4.6	6.0
Ash (% of DM)	4.8 (0.1)	4.4 (0.3)
Fiber (% of DM)	4.7 (0.2)	3.4 (0.4)
Calcium (% of DM)	0.9 (0.02)	0.9 (0.1)
Phosphor (% of DM)	0.5 (0.01)	0.5 (0.0)
Sodium (% of DM)	0.2 (0.0)	0.2 (0.0)
Vitamin E (mg/kg feed)*	added: 100 mg/kg diet	90.8 (2.3)
	Se-concentration (mg/kg diet) **	
Sodium selenite	0.40 (0.03)	0.28 (0.02)
Sodium selenite	0.60 (0.05)	0.64 (0.07)
L-SeMet	0.26 (0.04)	0.17 (0.01)
L-SeMet	0.43 (0.08)	0.43 (0.04)

Analysis methods: * HPLC, in form of α–tocopherol;**Mean (SD); 10 parallels measured with ICP-MSMS. Added Fe: sows´ feed – 153 mg, piglets´ feed – 260 mg; Added Cu: sows´ feed – 22 mg, piglets´ feed – 32 mg.

treatment (2 mg Meloxicam, intramuscular in the neck) at 2 weeks of age. All piglets were vaccinated at 3 weeks of age against Porcine circovirus 2 (Circovac®, Merial, France) according to the manufacturer's recommendations.

2.2. Experimental diets

All diets were produced at Felleskjøpet Rogaland and Agder, Stavanger, Norway. Maternal diets are described in Falk et al. [19]. During feed production there was a large variance in batches regarding Se concentration making it difficult to meet the planned dietary Se concentrations (NaSe-0.40 and -0.60, SeMet-0.26 and -0.43). The sows and their piglets received basic pelleted feed enriched with either selenite (Retosel, Selenium premix 1%, RETORTE Ulrich Scharrer Gmbh, Germany) or SeMet (supplied via the preparation Excential Selenium 4000, Orffa, Netherlands) at Se concentrations shown in Table 1. The Se concentration in the unsupplemented baseline diet was 0.04 mg Se/ kg in sow feed and 0.06 mg Se/kg in piglet feed.

2.3. Monitoring and sampling

Before first colostrum intake and at 5 days of age, piglets were weighed individually with an accuracy of 0.01 kg (EKS Premium 8006 GR-ST, Sweden). At the age of 24 and 31 days, piglets were weighed with an accuracy of 0.1 kg (KRUUSE Walk-on Scale, Jørgen Kruuse A/S, Denmark). The exact age of piglets was: 4.8 (0.8) days (herein: 5 days of age), 24.2 (1.5) days (herein: 24 days of age), 30.6 (1.3) days (herein: 31 days of age) and 37.6 (1.3) days (herein: 38 days of age).

Blood samples were isolated from all piglets weighing ≥1 kg before colostrum intake, and at ages of 5, 24, and 38 days. All blood samples were drawn from the *Vena jugularis externa* using the Vacuette® system (Greiner Bio-One, Austria). At the two first samplings, piglets were bled using needles sized 22Gx1″UTW and after that 20 G x ½"UTW (Venoject multi-sample needles, Terumo Medical, USA) sized needles were applied. According to the age and size of the animals, 3 or 6 mL Vacuette® Lithium Heparin tubes were used.

Tissue samples were obtained from stillborn piglets and piglets that died before colostrum intake (n = 5 per diet). Samples were isolated from kidney cortex, liver, myocardium, diaphragm, *Musculus longissimus dorsi* (LD), *M. semitendinosus* (ST) and *M. semitendinosus* (SM). Testicular tissue was collected during castration on day 14. Samples were stored at −20 °C until analysis.

2.4. Hematology and clinical chemistry

Blood was collected in Lithium Heparin tubes and subjected to a complete blood cell count using a multi-parametric hematological analysis (ADVIA 120 Hematology System, Siemens Healthcare GmbH) and after that centrifuged at 3500 *g* for 15 min (Megafuge 1.0 R, Heraeus SEPATECH, USA). Plasma samples were stored at −20 °C until analysis. The erythrocyte indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indicate the size and the hemoglobin concentration (Hgb) of erythrocytes and are calculated using the measured hematocrit (Hct) or Hgb, respectively, and divide it by the corresponding red blood cell count (RBC) or the Hct, respectively.

The clinical biochemistry analysis was conducted by applying the ABX Pentra400 analyzer (Horiba, France) including determination of GLDH (Roche Diagnostics, Norway), C-reactive protein (CRP, Randox Laboratories Limited, UK), as well as bilirubin, iron, gamma-glutamyl transferase (GGT), creatine kinase (CK), aspartate transaminase (AST), and lactate dehydrogenase (LDH) applying ABX, Horiba, France. The plasma concentrations of copper (Cu) were determined by atomic absorption spectrometry (AA300,Perkin Elmer, USA) at a wavelength of 324.8 nm. The plasma ceruloplasmin concentration (Cp) was determined with a biochemistry analyzer Cobas Mira (Roche) using a modification of the method described by Henry et al. [35].

The instruments were calibrated every day against controls. Controls have to fall into the range given in the kit (± 2 SD). Isolation, handling and analysis of samples were conducted in a randomized manner by the same personal throughout the trial period. An overview is now included in the supplemental material, Table A1.

2.5. Vitamin E measurements

Vacuum-packed feed samples and plasma samples in plastic tubes were stored at -80 and −20 °C, respectively, until overnight delivery in a freeze package to The Institute of Biochemistry, Faculty of Veterinary Medicine, University of Leipzig in Germany for vitE analysis. VitE concentrations (as α–tocopherol) were determined by high-performance liquid chromatography (HPLC) as described by Fuhrmann et al. [36].

The intra-assay variance was at 3.1 % and the inter-assay variance was at 7.4 % (QA-proceedures are viewed in Table A2, supplementary material).

2.6. Selenium measurements

Total Se in feed, plasma, and tissue samples was determined by inductively coupled plasma mass spectrometry (ICP-MSMS; Agilent 8800 or 8900, Japan) following similar methodology as previously applied [18,19,37]. Before microwave acid digestion (UltraClave system), ten feed subsamples per diet were finely ground and homogenized, tissue samples were freeze-dried, and enriched ^{74}Se (> 99.9%) was added to feed and tissues as an internal standard. Plasma samples were diluted (1:10 V:V) with a mixture of butanol, EDTA, NH_3, and Triton X-100 and also spiked with enriched ^{74}Se. Calibration standards were prepared from a Se ICP reference solution (Inorganic Ventures), and method blanks and certified reference materials (1567a wheat flour and 1570a spinach, NIST, USA; ERM® BD150 skimmed milk powder, JRC, Belgium; Seronorm™ L-1 and L-2 serums, SERO, Norway) were prepared and analyzed in the same manner as the samples. The accuracy of the method proves most acceptable since all measured concentrations were within 1.5% of certified values. The general ICP-MSMS operating parameters can be found in Falk et al. [19].

2.7. Selenium speciation in plasma

The distribution of Se biomolecules in plasma was determined by high-performance liquid chromatography (HPLC; Agilent HP1260 liquid chromatograph, Agilent Technologies Inc., USA) in tandem with ICP-MSMS (Agilent 8800, Japan) in time-resolved analysis mode [19]. Subsamples of thawed, homogenized plasma samples were ultrafiltrated using 3 kDa centrifugal filters (Merck, USA). Low (< 3 kDa, selenoaminoacids) and high (> 3 kDa, selenoproteins [Sel]) molecular weight Se-containing biomolecules were recovered in the filtrate and retentate fractions, respectively. Two different column set-ups under isocratic elution conditions were used to quantify the different Se biomolecules within each fraction. The amino acid SeMet was determined using an Atlantis T3 column with 7.5 % methanol mobile phase containing 0.1 % (V/V) of heptafluorobutyric acid (HFBA). SeMet has a retention time (t_R) of ~6.5 min. Se biomolecules (Sel glutathione peroxidase 3, GPx3; SelP; selenoalbumin, SeAlb) were separated using 1 mL HiTrap HP columns (GE Healthcare, Uppsala, Sweden) and ammonium acetate solutions as mobile phase [38]. With 0.05 M ammonium acetate, SelP and SeAlb are retained in the HiTrap Heparin and HiTrap Blue columns, respectively, whereas GPx3 is not retained (t_R ~2.5 min). SelP (t_R ~6.0 min) and SeAlb (t_R ~9 min) are then eluted sequentially from the columns by switching to 1.5 M ammonium acetate as mobile phase.

Chromatogram peaks associated to the Se biomolecules were integrated and their areas converted to Se concentration (in µg/L) using

calibration curves obtained from SeMet (seleno-L-methionine, ≥98% (TLC), Sigma Aldrich) standard solutions measured under the same elution conditions as for the Se biomolecules in the samples. The LOQ for each Se biomolecule was calculated as the Se concentration necessary to yield a net signal equal to 10 times the standard deviation of the background (i.e., 0.022 µg Se/L for SeMet, 0.039 µg Se/L for GPx3, and 0.041 µg Se/L for SelP and SeAlb). The precision of the method was < 1.5% for SeMet peaks and < 2.5% for GPx3, SelP and SeAlb peaks. Additionally, two certified reference materials (human serum BCR 637 and BCR 639; EC-JRC-IRMM, Belgium) were analyzed concerning total Se concentration and the reported Se biomolecules concentrations [39]. Measured concentrations of total Se and the four Se biomolecules were within ~4% for triplicate BCR samples, and the recovery of total Se and the sum of Se biomolecules respect to certified and reported values was 89–95% and 87–107%, respectively.

2.8. Statistics

The measurement for each response (blood variable) was modeled using a linear model (R *lm*; Version 1.1.383 – © 2009–2017 RStudio, Inc.) [40]. The explanatory variable *Group* was modeled as four different diets and *Gender* was modeled as two different sexes. The regressions (R *lm*) were repeated so that all diets were used as control. Data on hematology, clinical biochemistry and Se including Se biomolecules were compared between groups. Based on the Se concentrations in colostrum obtained during farrowing from the dams [19] of the litters studied here, we calculated two new sets of data. The estimated amounts of daily Se-intake via colostrum were determined using the Se concentration in colostrum as published by Falk et al. [19] combined with the recommended minimum colostrum intake of 200 g [41] and labeled "Minimum colostral Se intake". Also, the percentage of Se biomolecules on the total plasma Se concentration was calculated for the sows' plasma (values published by Falk et al. [19]) and for the corresponding offspring plasma.

The significance level was set to $P < 0.05$. Since we tested the four feeding groups against each other for each variable, a Bonferroni correction was conducted, and the resulting Bonferroni critical value was 0.0083. Therefore, only $P < 0.0083$ were taken as significant.

Only variables giving significant results are mentioned in the sections *Results* and *Discussion*.

3. Results

3.1. Body weight

No body weight (BW) differences between groups were observed at birth, but at 5 days of age, piglets in group NaSe-0.40 had significantly lower BW than those from group NaSe-0.60. At days 24 and 31, BWs in offspring from NaSe-0.40 fed sows were lower than in all the other groups (Table 2).

3.2. Hematology

At birth, piglets from sows fed SeMet-0.43 had lower RBC, Hgb and Hct, whereas the red cell distribution width (RDW) was higher compared with the group NaSe-0.40 (Table 3). In 38-days old piglets, the Hgb, Hct, MCH and MCV values were higher in piglets originating from NaSe-0.60 fed sows compared with the offspring from sows in the other diet groups.

3.3. Clinical biochemistry and VitE

The activity of GGT was higher in neonatal and 5-days-old piglets from sows receiving SeMet-0.26 compared with those from sows receiving NaSe-0.40 (Table 4). The activities of AST and LDH were lower in 38-days-old piglets from sows receiving SeMet-0.43 compared with that in piglets from the NaSe-0.60 fed sows.

The vitE level was not significantly different between piglet groups, but RBC counts as measured during the complete suckling period were significantly inversely correlated with vitE concentration in plasma (data not shown).

Table 2
Body weight (kg; mean ± SEM) from birth until weaning time and estimated amounts of Se intake (µg Se/day; mean ± SEM).

Diet	Body weight[#]	Estimated amount of Se intake[##]	
At birth	kg	n	µg Se/day
NaSe-0.40	1.50 ± 0.04^{a}	7	32.57 ± 2.21^{a*}
NaSe-0.60	1.61 ± 0.04^{a}	8	37.50 ± 4.20^{a}
SeMet-0.26	1.55 ± 0.03^{a}	7	48.57 ± 4.69^{ab}
SeMet-0.43	1.54 ± 0.03^{a}	8	58.25 ± 4.56^{b}
At day 5			
NaSe-0.40	2.33 ± 0.07^{a}		
NaSe-0.60	2.61 ± 0.06^{b}		
SeMet-0.26	2.52 ± 0.06^{ab}		
SeMet-0.43	2.40 ± 0.06^{ab}		
At day 24			
NaSe-0.40	6.82 ± 0.16^{a}		
NaSe-0.60	7.95 ± 0.21^{b*}		
SeMet-0.26	7.95 ± 0.19^{b*}		
SeMet-0.43	7.68 ± 0.16^{b*}		
At day 31			
NaSe-0.40	8.49 ± 0.21^{a}		
NaSe-0.60	9.89 ± 0.31^{b*}		
At day 5	10.12 ± 0.25^{b*}		
NaSe-0.40	9.36 ± 0.23^{ab}		

[#] Number piglets weighed were n = 44 (NaSe-0.40); n = 47 (NaSe-0.60); n = 48 (SeMet-0.26); n = 60 (SeMet-0.43). Means within a column without a common superscript differ significantly ($P < 0.0083$). *$P < 0.001$. [##]Calculated based on the recommended minimum colostrum intake of 200 g/d as published by Devillers et al. [41].

3.4. Plasma concentration of total Se and Se related biomolecules

Both Se source and Se level in maternal diets influenced the Se plasma concentrations in piglets (Fig. 1), resulting in generally higher plasma Se concentrations in the offspring from sows supplemented with SeMet. The piglets from SeMet-supplemented sows had a higher "estimated colostral Se intake" compared with those from sows supplemented with selenite (NaSe-0.40 and NaSe-0.60; Table 2).

Se-dose-related plasma concentrations were observed for GPx3, SeAlb and SeMet in piglets from sows fed SeMet enriched diets (Fig. 2). Mostly, these effects were also combined with a Se source effect in that highest concentrations were found in groups fed SeMet.

Concerning relative concentrations of Se biomolecules to total Se in plasma from neonatal and 5-daysold piglets from sows fed with SeMet, SelP accounted for less plasma-Se compared with the offspring from selenite fed sows (Table 5). The percentage GPx3 was higher in neonatal and 5-days-old piglets originating from sows fed with SeMet versus those from sows fed with selenite-enriched diets.

3.5. Selenium concentration in organ samples from perinatal piglets

The tissue Se concentrations (Table 6) were as follows: kidney cortex > liver > myocardium > skeletal muscles ≥ testicular tissue. Piglets from groups fed SeMet showed dose-related and significantly higher Se concentrations in testicular tissue than those fed with selenite. The myocardium, diaphragm, and ST had higher Se concentrations in piglets from groups fed SeMet than those fed NaSe. No significant differences in Se concentrations between groups were detected in LD, SM nor in kidney or liver.

Table 3
Hematological results during the trial period (mean (SD)) for each diet group and all groups.

	NaSe-0.40	NaSe-0.60	SeMet-0.26	SeMet-0.43	All groups	AverageRange	Reference	Average Ref.range	Reference
At birth	n = 16	n = 18	n = 14	n = 16	n = 64	1 day			
RBC	6.1 (0.6)^{b}	5.8 (0.5)^{ab}	5.8 (0.6)^{ab}	5.3 (0.6)^{a}	5.7 (0.6)	5.3 4.3-6.4	Thorn [137]		
Hgb	120.6 (11.1)^{b}	118.3 (12.2)^{ab}	115.2 (9.6)^{ab}	107.9 (14.6)^{a}	115.6 (12.7)	105 84-123	Thorn [137]		
Hct	39.2 (3.5)^{b}	38.1 (3.9)^{ab}	37.7 (3.8)^{ab}	34.8 (4.2)^{a}	37.5 (4.1)				
MCV	64.3 (3.8)^{a}	65.0 (3.0)^{a}	65.0 (2.0)^{a}	66.1 (2.7)^{a}	65.4 (3.0)	67 57-71	Thorn [137]		
MCH	19.8 (1.3)^{a}	20.5 (0.9)^{a}	19.9 (0.6)^{a}	20.4 (0.9)^{a}	20.2 (1.0)	20 18-21	Thorn [137]		
MCHC	308.2 (9.5)^{a}	310.4 (7.2)^{a}	306.0 (8.5)^{a}	309.2 (8.2)^{a}	308.6 (8.3)	305 289-313	Thorn [137]		
RDW	14.4 (0.7)^{a*}	14.4 (0.5)^{a}	14.6 (0.7)^{ab}	15.2 (0.7)^{b}	14.6 (0.7)				
day 5	n = 15	n = 19	n = 14	n = 14	n = 62	6 days			
RBC	4.5 (0.4)$^{b\hat{}}$	4.1 (0.4)^{ab}	4.1 (0.5)^{ab}	4.0 (0.6)^{a}	4.2 (0.5)	4.0 3.4-4.7	Thorn [137]		
Hgb	93.2 (5.8)^{a}	90.6 (10.0)^{a}	88.9 (8.4)^{a}	87.1 (12.6)^{a}	90.1 (9.5)	80 64-94	Thorn [137]		
Hct$^{\#}$	30.6 (2.1)^{a}	28.7 (3.3)^{a}	28.9 (3.4)^{a}	27.9 (3.9)^{a}	29.0 (3.3)				
MCV	67.7 (4.6)^{a}	69.4 (3.9)^{a}	69.8 (3.3)^{a}	69.5 (4.3)^{a}	69.1 (4.0)	67 60-74	Thorn [137]		
MCH	20.6 (1.4)^{a}	21.9 (1.2)^{b}	21.6 (1.1)^{ab}	21.7 (1.8)^{ab}	21.5 (1.5)	20 17-23	Thorn [137]		
MCHC	305.1 (8.9)^{a}	315.8 (9.3)^{b}	309.4 (12.2)^{ab}	312.5 (12.1)^{ab}	311.0 (11.1)	291 264-309	Thorn [137]		
RDW	21.5 (2.0)^{a}	22.9 (2.5)^{a}	23.4 (1.8)^{a}	22.7 (2.5)^{a}	22.6 (2.3)				
day 24	n = 4	n = 7	n = 9	n = 10	n = 30	20 days		Ca. 20 days	
RBC	6.5 (0.4)^{a}	6.3 (0.6)^{a}	6.3 (0.5)^{a}	6.1 (0.4)^{a}	6.3 (0.5)	4.9 4.4-5.3	Thorn [137]	6.0 4.8-7.3	[138]
Hgb	117.0 (21.4)^{a}	120.9 (13.6)^{a}	111.3 (9.6)^{a}	105.0 (9.8)^{a}	112.2 (13.4)	102 90-112	Thorn [137]	115 93-136	[138]
Hct	36.9 (4.7)^{ab}	37.9 (4.3)^{b}	36.7 (3.0)^{ab}	32.9 (2.9)^{a}	35.7 (3.9)			29 (6) 16-41$^{\#\#}$	[139]
MCV	56.0 (5.9)^{a}	59.9 (4.7)^{a}	58.2 (6.5)^{a}	54.2 (4.3)^{a}	57.0 (5.5)	76 70-82	Thorn [137]	66 53-79	[138]
MCH	17.9 (2.7)^{a}	19.0 (1.3)^{a}	17.7 (2.1)^{a}	17.4 (1.7)^{a}	17.9 (1.9)	21 19-23	Thorn [137]	19.5 15.0-23.0	[138]
MCHC	322.1 (28.4)^{a}	319.1 (13.1)^{a}	303.6 (14.9)^{a}	319.8 (11.6)^{a}	315.1 (16.8)	276 260-290	Thorn [137]	295 275-317	[138]
RDW	19.1 (1.4)^{a}	19.9 (2.6)^{a}	22.0 (2.9)^{a}	22.5 (4.1)^{a}	21.3 (3.3)			18.2 14.3-26.0	[138]
day 38	n = 15	n = 16	n = 14	n = 15	n = 60	36 days		6-weeks	
RBC	6.9 (0.3)^{a}	7.2 (0.5)^{a}	7.3 (0.7)^{a}	7.2 (0.4)^{a}	7.1 (0.5)	6.2 5.9-6.8	Thorn [137]	7.31 5.52-9.11	[140]
Hgb	114.5 (10.8)^{a*}	127.2 (8.0)^{b}	117.6 (5.9)^{a}	117.9 (7.7)^{a}	119.5 (9.5)	121 113-133	Thorn [137]	107 88-127	[140]
Hct	36.9 (2.8)^{a*}	41.9 (2.9)^{b}	39.1 (2.2)^{a}	38.2 (2.5)^{a*}	39.1 (3.2)			35.5 28.3-42.7	[140]
MCV	53.6 (3.8)^{a*}	58.5 (3.4)^{b}	54.1 (4.1)^{a}	53.4 (3.8)^{a*}	55.0 (4.3)	64 62-68	Thorn [137]	48.9 38.4-59.3	[140]
MCH	16.6 (1.4)^{ab}	17.8 (1.4)^{b}	16.3 (1.6)^{a}	16.5 (1.3)^{ab}	16.8 (1.5)	19.4 18.8-20.0	Thorn [137]	14.8 11.1-18.4	[140]
MCHC	310.0 (11.6)^{a}	304.0 (13.3)^{a}	300.9 (12.7)^{a}	308.9 (10.1)^{a}	306.0 (12.3)	305 280-320	Thorn [137]	302 279-324	[140]
RDW	20.3 (2.9)^{a}	18.5 (2.7)^{a}	20.7 (2.6)^{a}	20.7 (2.2)^{a}	20.0 (2.7)			24.4 16.4-32.3	[140]

Number given for n is the number of piglets considered at each time point. Means within a column without a common superscript differ significantly ($P < 0.0083$). $\hat{}P = 0.0087$. *$P < 0.001$. $^{\#}$Gender *female* higher by trend ($P = 0.0083$). $^{\#\#}$Pigs age in this publication: 30 days [139].
Units: RBC (x10^{12}/L), Hgb (g/L), Hct (%), MCV (fL), MCH (pg), MCHC (g/L), RDW (%).

4. Discussion

Results presented herein from piglets were obtained from sampling before (at birth) and after colostrum intake (5 days of age). Sampling at 24 days of age was conducted to demonstrate the piglets' status at an internationally commonly practiced weaning age, and at 38 days to present values from piglets experiencing post-weaning stress as piglets in this study were weaned at 33.6 (1.3) days which corresponds with the Norwegian average of 33.3 ± 0.3 days (2011–15) [42].

4.1. Performance

During the suckling period, piglets originating from sows fed SeMet gained more BW than piglets in group NaSe-0.40, which is in line with observations made by Zhan et al. [43], who applied selenite and DL-SeMet at a dietary level of 0.3 mg Se/kg diet. Weight gain is an indicator of well-being, vitality, and healthiness of neonates of many species [44]. However, Mahan and Kim [45], Mahan and Peters [46] and Quesnel et al. [47] used selenite and selenized yeast (Se yeast) as source of dietary Se at levels up to 0.4 mg Se/kg diet and studied sows and their offspring until weaning at 14 and 21 days of age or until 6

Table 4
Clinical biochemical and vitE measurements from birth until 38 days of age (mean ± SEM).

	NaSe-0.40	NaSe-0.60	SeMet-0.26	SeMet-0.43	AverageRange	Reference	Average Ref.range	Reference
At birth								
VitE[#]	0.31 ± 0.02^{a}	0.24 ± 0.02^{a}	0.31 ± 0.01^{a}	0.31 ± 0.03^{a}				
GGT (U/L)	79.6 ± 8.7^{a}	114.6 ± 10.6^{ab}	121.2 ± 11.1^{b}	115.0 ± 15.8^{ab}				
AST (U/L)	29.3 ± 4.7^{a}	27.7 ± 2.2^{a}	30.8 ± 4.7^{a}	48.0 ± 9.2^{a}				
LDH (U/L)	375.7 ± 33.7^{ab}	335.3 ± 25.7^{a}	411.0 ± 72.4^{ab}	540.3 ± 64.3$^{b\hat{}}$				
day 5								
VitE[#]	5.48 ± 0.57^{a}	5.69 ± 0.48^{a}	4.50 ± 0.34^{a}	4.50 ± 0.31^{a}				
GGT (U/L)	46.7 ± 5.2^{a}	59.0 ± 4.6^{ab}	67.3 ± 6.5^{b}	48.0 ± 3.3^{ab}				
AST (U/L)	39.5 ± 2.1^{a}	40.0 ± 2.2^{a}	36.7 ± 4.8^{a}	33.6 ± 2.5^{a}				
LDH (U/L)	629.3 ± 46.8^{a}	521.6 ± 17.8^{a}	476.7 ± 16.5^{a}	553.4 ± 56.7^{a}				
day 24							Ca. 20 days	
VitE[#]	2.08 ± 0.10^{a}	1.84 ± 0.11^{a}	1.99 ± 0.33^{a}	1.24 ± 0.17^{a}				
GGT (U/L)	43.8 ± 6.2^{a}	48.3 ± 4.9^{a}	49.1 ± 3.4^{a}	40.0 ± 1.3^{a}			35.0 14.0-64.0	[138]
AST (U/L)	44.3 ± 3.9^{a}	53.7 ± 8.9^{a}	45.8 ± 5.6^{a}	45.6 ± 4.6^{a}			38.3 18.0-83.5	[138]
LDH (U/L)	596.5 ± 42.6^{a}	636.0 ± 47.1^{a}	573.7 ± 52.5^{a}	553.4 ± 15.3^{a}				
day 38					Ca. 41 days**		6-weeks	
VitE[#]	1.06 ± 0.11^{a}	0.80 ± 0.05^{a}	0.95 ± 0.09^{a}	0.75 ± 0.12^{a}	1.6 (0.6) 0.4-4.1	[101]		
GGT (U/L)	56.1 ± 6.6^{a}	62.6 ± 5.1^{a}	61.0 ± 2.1^{a}	52.4 ± 3.2^{a}			57 33-94	[140]
AST (U/L)	46.1 ± 5.6^{ab}	54.1 ± 3.4^{b*}	44.3 ± 5.6^{ab}	29.7 ± 2.4^{a}			44 12-111	[140]
LDH (U/L)	690.7 ± 26.5^{ab}	776.0 ± 53.8^{b}	626.7 ± 25.1^{a}	617.9 ± 29.2^{a}				

< LOQ: underneath the limit of quantification.
Means (n = 7) within a row without a common superscript differ significantly ($P < 0.0083$). ˆP = 0.00838. *P < 0.001.
[#] Values from VitE (α-tocopherol; μg/mL) analysis are presented with two decimals due to low values. **Values detected in samples isolated 4 days after weaning [101].

weeks post-weaning. They observed no marked effect of the Se-source on postnatal growth of piglets [45–47]. In comparison to pure SeMet formulations Se yeast contains different Se compounds whereof selenomethionine accounted for 54–74% [48–50]. As reported by Falk et al. [19], a significantly higher average daily feed intake (ADFI) was observed in the corresponding dams fed with SeMet-0.26 and SeMet-0.43 diets compared with those fed with NaSe-0.40 or NaSe-0.60 diets from 13 days post-farrowing and throughout the lactation period. Furthermore, there was more total Se in colostrum and milk from SeMet-supplemented sows compared with those fed selenite enriched diets [19]. However, also piglets from sows fed NaSe-0.60 gained more BW than those in the group NaSe-0.40. This indicates that a level of 0.6 mg Se/kg from selenite and both levels of SeMet in the dams' diet have accelerated the offspring's growth. The influence of maternal ADFI was

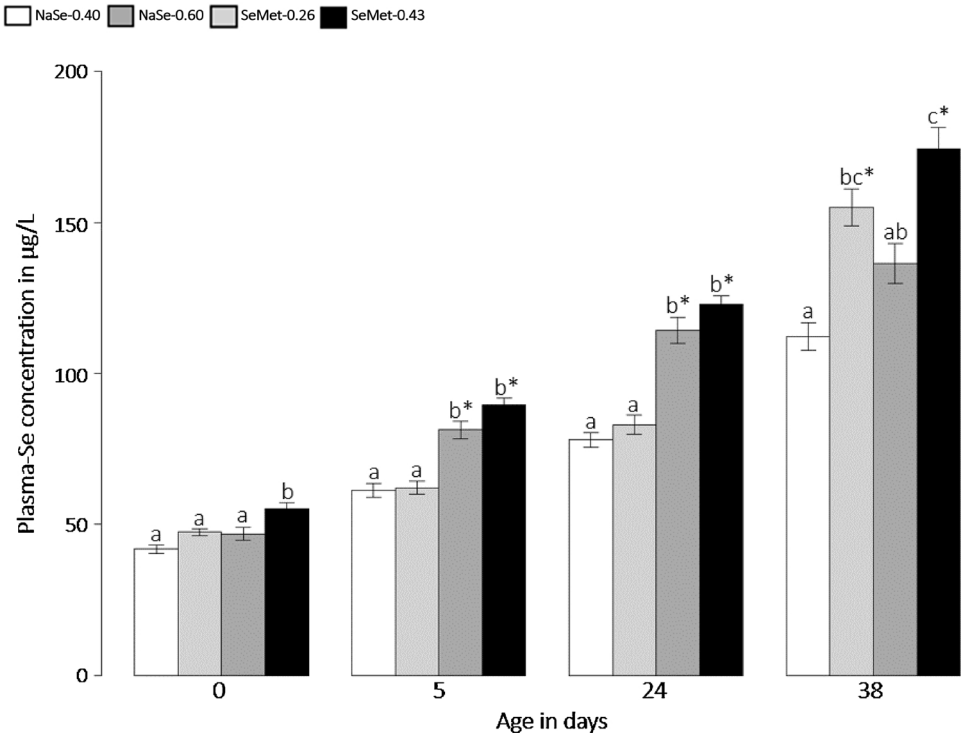

Fig. 1. Total selenium concentrations in plasma of piglets originated from sows fed with sodium selenite (NaSe-0.40, NaSe-0.60) and L-selenomethionine (SeMet-0.26, SeMet-0.43) enriched diets sampled at birth and 5, 24 and 38 days of age (n = 15/15/6/9, 15/15/6/8, 15/15/7/9, 12/15/7/11 for each diet and each time point). Bar plots (mean Se concentration in μg/L ± 95 confidence interval) at one time point without a common superscript differ significantly ($P < 0.0083$) and * superscript means $P < 0.001$.

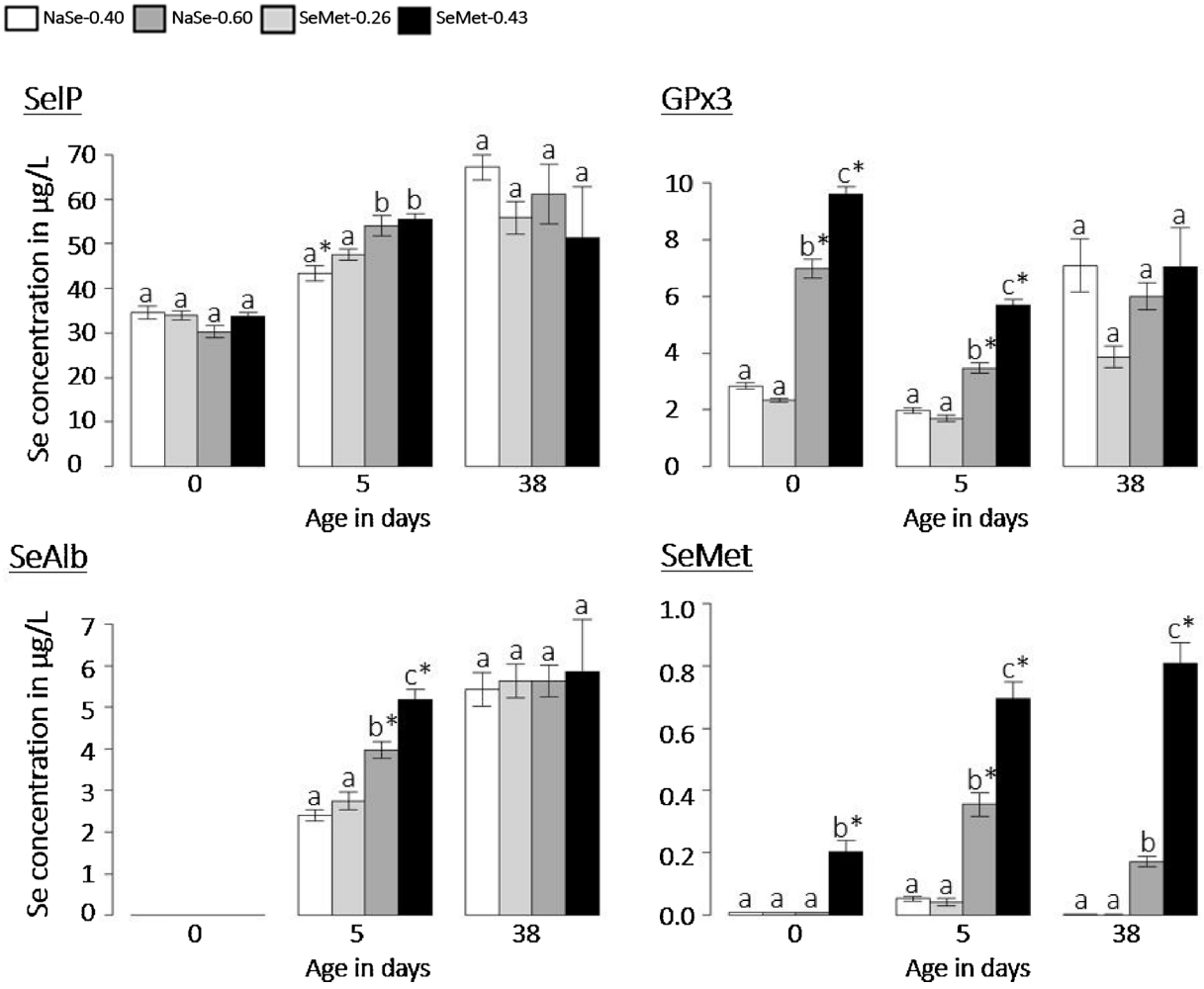

Fig. 2. Distribution of Se biomoecules in plasma of piglets originated from sows fed with sodium selenite (NaSe-0.40, NaSe-0.60) and L-selenomethionine (SeMet-0.26, SeMet-0.43) diets at 0, 5 and 38 days of age (n = 15/15/9, 5/15/9, 15/15/9, 15/15/11 for each diet and each time point). Bar plots represent the mean Se concentration associated to each Se biomulecule (in µg/L) ± 95 confidence interval. Bars at one time point without a common superscript differ significantly ($P < 0.0083$) and * superscript means $P < 0.001$.

Table 5
Percentage of selenium (Se) associated to Se biomolecules with respect to the total Se concentration in plasma from sows at farrowing[#] and in corresponding piglets' plasma prior to colostrum intake and on days 5 and 38 of age (mean (SD)).

	% of Se							
	n	NaSe-0.40	n	NaSe-0.60	n	SeMet-0.26	n	SeMet-0.43
Total Se; piglets vs. sows[#]	5	22.2 (6.0)^{a*}	5	29.3 (3.9)^{b}	5	24.0 (5.2)^{ab}	5	29.6 (5.1)^{b}
Sows[#]	5	51.0 (7.3)^{a}	5	53.0 (7.6)^{a}	5	49.0 (4.5)^{a}	5	44.0 (5.3)^{a}
Piglets (at birth[#])	15	82.4 (4.7)$^{c*, B*}$	15	71.6 (3.9)$^{b*, B*}$	15	65.0 (6.2)$^{a, B*}$	14	61.5 (4.3)$^{a, B*}$
Piglets (day 5)	15	71.4 (11.2)$^{bc, AB}$	15	75.8 (5.5)$^{c*, B*}$	15	66.5 (5.5)$^{ab, B*}$	15	62.3 (4.9)$^{a, B*}$
Piglets (day 38)	7	63.6 (9.9) $^{b*, A}$	8	35.7 (7.7) $^{a, A}$	7	45.5 (14.4) $^{ab, A}$	5	36.8 (11.2)$^{a, A}$
SeAlb								
Sows[#]		8.4 (1.6)^{a}		8.3 (1.2)^{a}		6.9 (0.63)^{a}		7.7 (1.7)^{a}
Piglets (at birth[#])	15	< LOD^{A}	15	< LOD^{A}	15	< LOD^{A}	14	< LOD^{A}
Piglets (day 5)	15	4.0 (0.8)$^{a, B}$	15	4.4 (1.2)$^{a, B*}$	15	4.9 (0.6)$^{b, B*}$	15	5.8 (1.1)$^{c, C*}$
Piglets (day 38)	6	4.8 (0.5)$^{a, C}$	8	3.7 (1.1)$^{a, B*}$	7	4.2 (1.1)$^{a, B*}$	5	4.2 (1.2)$^{a, B*}$
GPx3								
Sows[#]		*not measured*						
Piglets (at birth[#])	15	6.8 (0.9)$^{b, B*}$	15	5.0 (0.9)$^{a, B*}$	15	15.1 (1.9)$^{c*, B*}$	14	17.7 (2.4)$^{d*, C*}$
Piglets (day 5)	15	3.3 (0.8)$^{a, A}$	15	2.7 (0.7)$^{a, A}$	15	4.3 (1.1)$^{b, A}$	15	6.4 (0.9)$^{b*, B}$
Piglets (day 38)	6	6.0 (2.0) $^{b, B*}$	8	2.5 (1.0)$^{a, A}$	7	4.5 (1.3) $^{ab, A}$	7	3.4 (1.8)$^{a, A}$

Means within a row or within a column without a common superscript differ significantly ($P < 0.0083$). Capital letters notify significant differences between time points, whereas small types indicate significant differences between feeding groups at the same time point. *$P < 0.001$.

[#] Results are based on measurements of total Se and Se speciation in plasma samples collected at farrowing pubished in Falk et al. [19].

Table 6
Selenium concentration in tissues (mg/kg dry matter) isolated from stillborn piglets or newborn piglets that died before colostrum intake (n = 5 per diet; mean (SD)).

Diet group	Kidney cortex	Liver	Myocardium	Diaphragm	LD[#]	SM[#]	ST[#]	Testicular tissue[##]
NaSe-0.40	3.12 (0.81)[a]	1.24 (0.41)[a]	0.81 (0.09)[a]	0.32 (0.09)[a]	0.28 (0.08)[a]	0.28 (0.10)[a]	0.29 (0.08)[a]	0.21 (0.02)[a]
NaSe-0.60	3.50 (0.79)[a]	1.42 (0.36)[a]	0.76 (0.07)[a]	0.42 (0.13)[ab]	0.29 (0.06)[a]	0.30 (0.07)[a]	0.31 (0.06)[a]	0.24 (0.04)[ab]
SeMet-0.26	3.28 (0.46)[a]	1.28 (0.56)[a]	1.02 (0.14)[b]	0.50 (0.16)[ab]	0.33 (0.09)[a]	0.37 (0.17)[a]	0.39 (0.12)[ab]	0.29 (0.04)[b*]
SeMet-0.43	3.26 (0.44)[a]	1.60 (0.48)[a]	1.17 (0.12)[b*]	0.60 (0.11)[b]	0.40 (0.05)[a]	0.43 (0.07)[a]	0.48 (0.07)[b]	0.38 (0.02)[c*]

Means within a column without a common superscript differ significantly ($P < 0.0083$). *$P < 0.001$.
[#] *Musculus longissimus dorsi* (LD), *M. semimembranosus* (SM), *M. semitendinosus* (ST).
[##] measured in tissue isolated at day 14.

ambiguous.

Selenite has been shown to exert stimulatory effects on the growth of cells by yet incompletely characterized mechanisms [51]. Selenoproteins, as e.g. iodothyronine deiodinases (DIOs) and thioredoxin reductases (Txrnd), are essential for optimal growth [12,52–54]. Se is required for the conversion of thyroxin (T4) into the more active triiodothyronine (T3) via the DIOs [55]. Additionally, selenoperoxidases and Txrnd protect the thyroid gland from ROS produced during hormone synthesis [56]. For incorporation in selenoproteins, both selenite and SeMet have to be transformed to selenide (H_2Se), the precursor of selenocysteine (SeCys). The positive influence on piglets' growth observed with SeMet may be due to the Se being part of selenoproteins influencing e.g. the antioxidant system and the immune response. Furthermore, the amino acid SeMet is a building block of non-seleno- body proteins, promoting neonatal intestinal growth [57], increasing the acitivity of pancreatic enzymes [43] and influencing gene expression and methylation in the offspring [58]. In addition, the sulfurized equivalent of SeMet, methionine, is vital for the architecture and barrier function of the intestine and other intestinal functions including digestion, absorption, and metabolism of nutrients [59,60].

4.2. Hematology

Hematological parameters such as Hct, Hgb, RBC, and WBC, are used to assess the functional status of the oxygen carrying capacity and might also indicate responses of an organism to selenite [21,22]. However, piglets are prone to anemia [61] and Se has been shown to influence erythropoiesis [62,63].

Higher RBC counts as observed in neonatal and 5-days-old piglets from sows fed with NaSe-0.40 diet versus those from SeMet-0.43 supplemented sows might illustrate the immaturity of the hepatic transsulfuration/transselenation pathway (TS-pathway) in fetal and perinatal pigs [64,65] possibly impairing erythropoiesis in offspring from SeMet-supplemented sows. SeMet has to be transselenated before its Se can enter the selenoprotein synthesis pathway, and the activity of the hepatic TS-pathway has been shown to increase rapidly postnatal in humans and rats [66–68]. Furthermore, the duration of the pre-farrowing part of the feeding trial in the corresponding sows fed from 1 month prior to farrowing [19] might have had an impact. Selenite is metabolized to SeCys, the building blocks in selenoproteins, such as e.g. erythrocyte GPx [69], but not stored for later use [70]. In addition to the entrance in this pathway, SeMet, as the selenized analog of methionine, can be incorporated into non-selenoproteins like Hgb [71] and other body proteins. In transgenic mice, it has been shown that selenoproteins take part in the regulation of the erythropoiesis and maintain the redox homeostasis in erythroid cells [72]. However, the higher dietary concentration of selenite in the sows fed with NaSe-0.60 diet might, after entering the fetal circulation, have had a negative influence on erythropoiesis due to the production of ROS during the selenite metabolism. Other authors have shown that metabolizing the rather strong oxidizing selenite can trigger endoplasmic reticulum stress due to the generation of ROS [21,22]. Based on results from gilts fed diets enriched with selenite at a level of 0.3 mg Se/kg, Dalto et al. [20] suggested, that an excess concentration of the highly toxic H_2Se could be formed in the embryo, damaging mitochondria and impairing the ATP synthesis. In summary, in the offspring from sows fed NaSe-0.60, SeMet-0.26 and SeMet-0.43 the erythropoiesis might have been impaired by either the ROS production or the immature TS-pathway.

Regarding the higher Hgb-concentration in neonatal piglets from sows fed selenite-supplemented feed, we do again suggest a relationship with the above-mentioned immature TS-pathway [64,65]. The expansive erythropoiesis of the fetal liver is thought to be mechanistically similar to stress erythropoiesis [73] and selenoproteins have been shown to regulate stress erythroid progenitors during stress erythropoiesis [62].

Higher Hgb, Hct, MCV and MCH values in 38-days-old piglets in the NaSe-0.60 fed group compared with those receiving one of the other three diets might indicate a better oxygenation of the pigs in the high selenite group. A direct link between the intracellular free Ca2+ concentration and the hemoglobin oxygen saturation has been reported in humans [74]. Observations reported for selenite-fed grower-finisher pigs in Falk et al. [18], feeding NaSe-0.60 to sows and their offspring might influence the synthesis of selenoproteins in the offspring involved in the regulation of the Ca^{2+}- homeostasis in the cell [75]. Membrane-bound selenoproteins, e.g. Sel I, K, N, S and T, have been shown to modulate the Ca^{2+}- flux [76,77].

4.3. Clinical biochemistry

We observed lower GGT-activity in neonatal and 5-days-old piglets from sows fed with NaSe-0.40 diet compared with the offspring from the other groups. GGT catalyzes the degradation of extracellular GSH to enable its *de novo* synthesis in the cell thus increasing the intracellular antioxidant level [78] and is thus involved in the antioxidant defense [79,80]. GSH is involved in various cellular processes including cell growth and proliferation [81,82] and the cellular GSH- level is responsive to the redox state of the intracellular environment as well as the growth state of the cell [83–85]. Experiments examining GSH levels in growing versus growth-arrested cells showed declining GSH-levels as cells approached quiescence [81,83]. Thus, the GGT-activity is possibly linked to the higher weight gain observed herein in the offspring from SeMet and NaSe-0.60 fed sows. In plasma from SeMet-0.26 fed sows, we could show that the activity of GGT increased stronger throughout the lactation period compared with the other groups [19] possibly related to increased metabolic demands because of, e.g., increased milk production.

Higher AST- and LDH-activity in the extracellular space in weaned piglets from NaSe-0.60 fed sows might indicate higher ROS-production in hepatocytes. AST-activity has been shown to be higher in weaned piglets and was suggested to indicate hepatic damage due to oxidative stress [86]. Selenite, but not SeMet, induced oxidative stress due to the production of ROS in cells with high metabolic activity [87], as shown by a significant increase of LDH-leakage [88].

4.4. Vitamin E

Due to ineffective placental transfer of vitE, the neonates' serum-concentrations are low [89,90], which is in line with results presented

herein. Malm et al. [91] and Mahan [89] showed that piglets receive vitE with colostrum and milk, explaining our observations on increased plasma-vitE-concentrations in older piglets. Our results showed an inverse correlation of the plasma-vitE-concentration with the RBC-counts from birth throughout the study. Erythrocytes are at higher risk of damage from peroxides than most other cells due to their high concentration of oxygen [92] and erythrocyte membrane fluidity (EMF) is enhanced by the presence of (poly)unsaturated fatty acids. In sheep erythrocytes, vitE is shown to act as a membrane-stabilizing agent, independently of its antioxidant properties [93]. In an in-vitro study, pig erythrocytes exhibited the lowest resistance to oxidative stress compared with ducks and chickens [94]. The liver preferentially incorporates α-tocopherol into lipoproteins that are released into the bloodstream for distribution to peripheral tissues [95]. α -tocopherol transfer protein selectively binds to hepatic α –tocopherol, transports it throughout the body and releases it into cellular membranes [96]. Erythrocytes are the major cellular component of blood, and EMF can be affected by oxidative stress [97]. Thus, the higher the count of needy cells the lower the plasma VitE-concentration.

A further genetic selection for rapid growth leading to increasing RBC counts might subsequently increase the incidence of vitE-deficiency related problems in swine production. This might explain survey results obtained by the Norwegian Meat and Poultry Research Center in 2011, stating an increasing occurrence of Se-deficiency related disorders in Norwegian pig production [98]. In addition, plasma-vitE- levels in our study decreased after weaning, as also shown by others [99–101], coinciding with a higher occurrence of Se deficiency related Mulberry heart disease as described by several authors [102–105].

4.5. Plasma selenium concentration

The higher plasma Se concentration in piglets before colostrum intake from sows supplemented with dietary Se from SeMet is in line with results from Yoon and McMillan feeding Se yeast enriched feed [106] and from Finch et al. [107], who proved the existence of placental transport systems for neutral amino acids in pigs. Both selenite and SeMet [45] are transferred from the sow via the placenta to the fetus. In our study, there was a lack of a dose-response in the offspring from selenite supplemented sows, which is in line with results from others [108,109] proving that SeMet is more effectively transferred than selenite.

On day 5, lower plasma Se concentrations were observed in piglets from selenite fed sows compared with those in offspring from sows receiving SeMet-enriched diets, and a dose-response effect was only seen with increasing age. Based on Devillers et al. [41], we calculated the amount of Se potentially taken up by the piglets in our study and the results did not explain the absence of a dose-response effect on the Se plasma concentration in piglets from selenite fed sows. However, it might reflect that the Se supplementation of NaSe-0.60 fed dams was above the offspring's requirements.

In contrast to our results, Mahan [110], who added selenite or Se yeast at levels of 0.15 or 0.30 mg Se/kg diet, observed that serum Se levels in 7-days-old piglets were not influenced by the Se source but increased as the dietary Se level in sow feed increased. This might indicate a suboptimal Se supplementation in the sows in Mahan's study [111], at least in case of the offspring from sows fed with the lower dietary level of selenite (0.15 mg Se/kg). Also, recent literature has shown that there might be a large batch-to-batch variability for organic Se in Se yeast [48,111] and, as opposed to pure L-SeMet, Se yeast contains a mixture of selenocompounds [112]. Until day 14, the serum Se concentration in Mahan's study showed the most significant increase in piglets from sows fed with organic Se [110], which is in line with our observation in piglets aged 5 and 24 days. Mahan [110] concluded that organic Se is incorporated more effectively into sow milk proteins and had a 2.7-fold higher bioavailability than Se from inorganic sources.

4.6. Plasma selenobiomolecules

The sharp increasing activity of the hepatic TS-pathway postnatally [66–68] enables the use of Se from organic Se sources to a much higher degree and possibly explains our results on significant higher plasma SelP concentrations in 5-days-old piglets from SeMet-supplemented sows. As shown previously, Se from SeMet fed to the corresponding sows is transferred to a significantly higher degree to colostrum and milk than Se from selenite [19]. There was a lack of significant dosage effect on the plasma SelP concentrations in piglets, whatever age, from sows receiving Se at two different dietary levels. This might indicate that the supply with Se in the piglets originating from sows fed with NaSe-0.60 diet is above the requirements since the saturation of SelP in plasma is accepted as a biomarker of determining the optimum supply of Se [113]. At the same time, maternal dietary supplementation with selenite at a level of 0.4 mg Se/kg possibly met the fetal requirements for the production of SelP around parturition. Higher percentages of SelP in total Se in plasma of piglets compared with those in mature sows presented herein highlight the importance of this selenoprotein in young, fast-growing pigs as an antioxidant and for Se transport, as well as for its distribution throughout the body. Keeping in mind the immature hepatic TS pathway in fetal and perinatal pigs, this might also support the suggestion of cysteine as a "conditionally" essential amino acid for both fetus and neonate [67,68], explaining at the same time the suggested decomposition of maternal SelP in the murine placenta with subsequent release of SeCys into the fetal circulation [114]. The existence of the TS-pathway was proven for the human placenta [115,116].

Our results on GPx3 seem to counteract the discussion on the immature TS-pathway, but GPx3 is produced in the kidney, and this organ has a stable TS-pathway activity from early stages of fetal development, though, late in pregnancy, this acitivity is lower than in the liver [66]. Katzer et al. [117] suggested that oxidative stress during labor leads to an elevation of GPx3 and other antioxidants in the human fetal circulation, protecting the newborn from severe impairment. This is in line with our observations on GPx3 in plasma from SeMet fed sows at birth versus later in life. In line with Mahan et al. [118] piglets in our study were low in total plasma Se at birth. However, since maternal dietary Se from SeMet increased the antioxidative selenoprotein GPx3 in the corresponding piglets' plasma to a high degree, we suggest that maternal dietary SeMet increased the antioxidant capacity in newborn piglets' plasma and in that of 5-days-old compared with the offspring from sows receiving selenite-supplemented diets. The results on Se biomolecules as well as on the total plasma Se concentration raise the question if maternal dietary selenite at the level of 0.6 mg Se/kg feed is above the piglets' requirements for synthesis of the extracellular selenoproteins SelP and GPx3. These two Se biomolecules contained together on average about 86% of the Se in plasma of newborn piglets in this study, but the percentage was lower in offspring from NaSe-0.60 fed sows. Placental release of SelP-derived SeCys into the fetal circulation as suggested in mice [114] in combination with the placental transport of SeMet [45,114] would explain the dose-response effect seen for the GPx3 concentration in plasma from newborn piglets' originating from the SeMet supplemented sows. At farrowing, no source or dose-response effect was observed on the plasma SelP concentration in the corresponding sows [19] possibly due to increased maternal-fetal transfer and, during lactation, Se transport to the mammary gland as described in mice [119].

An increase of SeAlb from non-detectable amounts before colostrum intake to on average 5.2 μg/L at day 5 was observed for piglets in all four feeding groups. Rootwelt et al. [44] showed that blood levels of albumin in piglets increased more than 3-fold during the first day of life. In contrast to selenite, Se in the form of SeMet is incorporated in the amino acid chain of albumin thus forming SeAlb [120]. This explains the significantly higher values in piglets originating from sows fed with SeMet enriched feed. The detection of SeAlb in piglets from

sows receiving selenite supplemented diets might be explained by an increased protein turnover in the dam during pregnancy, as found in pregnant humans [121], followed by transport of released maternal SeMet to the fetus originating from pre-trial maternal diets. The absence of significant differences in the plasma concentrations of SelP, GPx3, and SeAlb on day 38 might mirror weaning stress. Events, like weaning with subsequent lack of maternal milk and loss of maternal bonding, mixing of different litters, transportation to growing-finishing farms, changing housing conditions and especially due to reduced feed intake can have a negative impact on the pig [122]. Thus, reduced Se supply as a result of reduced feed intake after weaning [108] and thereby a disturbance of the intestinal function [123] combined with higher stress levels [122] may have resulted in a lack of differences between groups regarding SelP, GPx3 and SeAlb.

SeMet, both as free amino acid and bound in, e.g., selenoalbumin, provides the basis for a more stable Se status via the TS-pathway. Throughout this study, plasma SeMet concentrations were highest in plasma from piglets originating from sows fed the SeMet-0.43 diet as expected due to proven maternal-fetal Se transfer in pigs and mice [45,114], its dose-dependent occurrence in the corresponding porcine colostrum and milk [19] and intestinal absorption.

4.7. Tissue selenium concentrations

Higher Se levels were observed in the myocardium and selected skeletal muscle tissues from piglets originating from sows receiving dietary SeMet compared with those from sows fed selenite supplemented feed. This is in line with Fortier et al. [109] and Svoboda et al. [124] who stated that maternal dietary SeMet is to a high degree transferred to the porcine progeny. In line with results from Mahan et al. [108], kidney Se concentrations were low in neonatal pigs studied herein compared with those in older pigs as published by Falk et al. [18]. Increasing kidney Se concentrations during growth reflect the kidneys function in Se excretion via the urine [45]. In contrast to our results, Svoboda et al. [124], Ma et al. [125] and Mahan and Peters [46] detected significant higher liver tissue Se concentrations in piglets from sows fed organic Se in the form of Se yeast from before breeding. Our trial in the corresponding sows did comprise a shorter period. Intake of SeMet over a more extended period establishes a steady state enabling the release of SeMet from maternal body proteins during protein turnover which occurs continuously [126]. Our results on heart and skeletal muscle tissue Se in offspring from sows fed SeMet prove the maternal-fetal transfer of SeMet and its incorporation into fetal proteins theoretically providing the basis for more stable plasma Se concentrations in piglets based on protein turnover during growth.

A clear dose and Se source effect was found for testicular Se concentrations. In testes, Se is of fundamental importance, as Se deficiency reduces testicular mass, changes morphology, and causes flagellar defects in sperms [127,128]. Se-deficiency causes oxidative stress in testes due to the diminished antioxidant property of this element as part of GPx [129]. Supranutritional levels of selenite can cause severe abnormalities in sperms due to increased oxidative stress [129–131].

In conclusion, the application of SeMet to the corresponding dams led to SeMet storages in the piglets body during intrauterine growth enabling a stable Se homeostasis in the neonates as indicated by the Se levels measured herein in tissue from the heart and skeletal muscles (Table 6).

Until 2016, diets for porcine offspring in Norway were mainly enriched with selenite. Assuming a basal Se level of < 0.1 mg Se/kg diet and taking into account the maximal allowed dietary Se level of 0.5 mg/kg diet [132], Norwegian pigs received at least 0.3 mg Se/kg diet from selenite. Selenite, though a suitable Se substrate for the formation of selenoproteins, cannot be stored for later use [70]. Selenite has been shown to stimulate lipid peroxidation and its biotransformation to H_2Se decreases antioxidative reserves [22,133]. However, SeMet can increase selenoenzyme activity, and it can be stored in tissues, giving it a slower whole-body turnover rate and allowing it to support higher tissue Se concentrations than inorganic Se [48]. This second metabolic pathway may confer protection against excessive amounts of H_2Se and prevent toxicity mediated through ROS from excessive intakes. Schrauzer [126] pointed out that supplementation with inorganic Se salts deprived the growing infant of the benefits only provided by SeMet and suggested that Se should be supplemented in the form occuring naturally in foods [134]. The L-isomer of SeMet is a major natural form of Se. Thus, synthetic LSeMet is an appropriate supplemental form of Se [134].

In line with other authors, we showed that the concentrations of Se [108] and vitE [25] are low in newborn piglets [135,136] and that plasma vitE decreased after weaning [101]. Thus, both the positive influence on selenoproteins in terms of protection against oxidative stress and the storage of SeMet in tissues are advantageous features reducing the risk of entering a marginal or deficient Se status [48]. The use of selenite might lead to an additional decrease of antioxidants in the weaning period since this Se-containing compound will not be incorporated into proteins like SeMet.

5. Conclusion

By comparing SeMet 0.43 and NaSe0.40 it was obvious that SeMet was more effiecently transferred over placenta. After intake of colostrum and milk as well as concentrate feed the higher bioavailablity of SeMet compared with NaSe became more clear. Higher plasma levels of GPx3 in perinatal piglets, especially from dams receiving dietary SeMet, might increase their protection against birth-related oxidative stress. Moreover, findings in neonatal piglets from sows fed with dietary SeMet are consistent with a still immature TS-pathway. Several findings in sows fed with NaSe-0.60 diet and their offspring might reflect supranutritional supply with selenite. However, supplementation of maternal diets with selenite seemed to be favorable for prenatal erythropoiesis. Given these results, a combination of inorganic and organic Se sources in complete feed for pigs might be beneficial in diets for breeding sows in terms of both reproduction and the offspring's growth performance. Thus, further research should be conducted using a combination of these two chemical forms of dietary Se at dietary levels complying with current legislation.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.126439.

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