Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Production Animal Clinical Sciences
Section for Small Ruminant Research

Philosophiae Doctor (PhD)
Thesis 2022:10

Selenium requirement in Norwegian high-yielding pigs

Selenbehov hos norske høytytende griser

Michaela Falk

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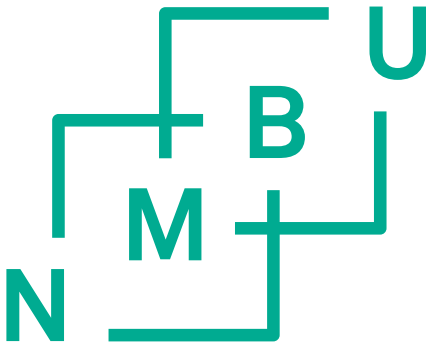

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Michaela Falk, Sandnes, December 2021

Abbreviations

ADG	Average daily weight gain
BW	Body weight
Cat	Catalase, *cat* denotes the corresponding gene
CI	95% confidence limit of mean
Cox	Cyclooxygenase, *cox* denotes the corresponding gene
DIO	Iodothyronine deiodinase, *dio* denotes the corresponding gene
DM	Dry matter
GGT	Gamma-(γ-)-glutamyl transferase
GLDH	Glutamate dehydrogenase
GPx	Glutathione peroxidase, *gpx* denotes the corresponding gene
GSH	Glutathione, reduced glutathione
H_2O_2	Hydrogen peroxide
H_2Se	Selenide
Hct	Haematocrit
Hgb	Haemoglobin
ICP-MSMS	Inductively coupled plasma - mass spectrometry
IFN-ɤ	Interferon-gamma, *ifnɤ* denotes the corresponding gene
IL	Interleukin, *il* denotes the corresponding gene
LPS	Lipopolysaccharide
Mg	Magnesium
$^{\bullet}O_2^-$	Superoxide
OH•	Hydroxyl radical
OH^-	Hydroxide
PUFA	Polyunsaturated fatty acids
RT-qPCR	Quantitative reverse transcription-polymerase chain reaction

R©	Free language and software environment for statistical computing and graphics
ROS	Reactive oxygen species
SD	Standard deviation S
SEM	Standard error mean
Se	Selenium
SeCys	Selenocysteine
Selenite	Sodium selenite (Na_2SeO_3; NaSe)
Seleno	Prefix in some selenogenes
SeMet	Selenomethionine, herein mainly L-enantiomer
Txnrd	Thioredoxin reductase, *txnrd* denotes the corresponding gene
Vit	Vitamin

Summary

Selenium (Se), one of the essential trace elements with respect to animal and human health, has a narrow range spanning from essentiality to toxicity. It unfolds its vital properties in living organisms as part of the 21st genetically encoded amino acid selenocysteine (SeCys). As SeCys in polypeptide chains of selenoproteins, Se is transported throughout the body and increases the reactivity of selenoenzymes compared to their Cys-analogues.

The main natural Se source in swine feed is grains. Norwegian inland soil is largely poor in Se and, therefore, the overall Se concentration in Norwegian cereal grains is low. To meet the pigs' Se requirements it is necessary to enrich swine feed with Se supplements. Since the 1980s in Norway, this has mainly been based on the addition of the inorganic Se source sodium selenite (selenite). Although commercial feed in Norway has been supplemented with concentrations close to the upper limits of 0.5 mg Se/kg feed according to the current European Union (EU) legislation, problems related to Se deficiency have continued to occur in pigs. The Norwegian feed industry has tried to counteract these challenges with high dietary doses of vitamin E (vitE) since there is no upper limit for vitE supplementation within the EU. Both Se, in selenospecies (Se species), and vitE possess antioxidant properties and deficiency might lead to oxidative stress-related problems. VitE and Se are interrelated in the prevention of several diseases in animals and man, and each can reduce signs of deficiency of the other.

The main objective of this project was to compare the effects of dietary L-selenomethionine (SeMet; organic Se compound) and selenite as Se sources on pig health. The secondary objectives were to investigate (a) whether the upper dietary Se limits set by the EU support healthy development of high-yielding, fast-growing pigs, (b) the Se uptake in bodily fluids and tissues of pigs at all ages, and (c) the influence of Se sources and dietary Se levels on the

expression of seleno- and non-selenogenes as well as several clinical parameters.

A descriptive Mulberry heart disease (MHD) study (**Paper I**) was undertaken to identify Se levels in a series of organs and tissues collected from acute dead pigs showing lesions consistent with MHD although receiving diets supplemented with Se and vitE according to actual EU legislation and recommendations. To address the above-mentioned objectives, two feeding trials were conducted, each including four diets. An isolated grower-finisher study (**Paper II**) included 24 weaned pigs. The following sow-offspring trial comprised 32 sows, studied from late gestation, through the farrowing and lactation period (**Paper III**), and the offspring's life until slaughter (until weaning included in **Paper IV**). The expression of a selection of genes in whole blood (**Papers II**), isolated before and after the intravenous administration of lipopolysaccharides (LPS), as well as in samples taken from *Musculus longissimus dorsi* (*M. long. dorsi,* only **Paper II**) during the grower-finisher period, was evaluated for eventual Se-source- or -level-related differences. Additionally, measurements of the total Se level were conducted in heparinized plasma, colostrum, milk, and several tissues (**Papers II-IV**). As the biochemical activity of Se compounds depends on the speciation of selenium in the body, information on the presence of different Se species is essential to explain eventual effect differences observed in sows (**Paper III**) fed selenite or SeMet at different dietary concentrations and in their offspring (**Paper IV**). Se speciation studies were performed using plasma samples from sows and offspring as well as colostrum and milk samples.

Results from the descriptive MHD study showed that mean Se concentrations in MHD cases were significantly lower in samples from the myocardium, in nine of ten skeletal muscles, gastric ventricle, duodenum, jejunum, caecum, liver, kidney, thymus, and skin compared with controls (**Paper I**). These results show that the inclusion of samples from skeletal muscles for chemical analysis might improve the MHD diagnosis.

In the grower-finisher-study (**Paper II**), in *M. long. dorsi* samples from pigs fed Se-supplemented feed (0.3 mg Se/kg of either selenite [NaSe], selenized yeast [Se yeast] or SeMet), the selenogenes *SelenoW* and *SelenoH*, both encoding thioredoxin-like selenoproteins (rdx-family), were expressed at a similarly higher level compared with controls without Se supplementation over a 64-days period. In samples from the same feeding groups, the genes encoding the enzymatic antioxidants GPx3 and superoxide dismutase 1 (SOD1; day 38) as well as the cytokine interferon-gamma (IFN-ɤ) and the enzyme cyclooxygenase 2 (Cox2, catalyses the prostanoid formation; day 64), were expressed at lower levels than in muscle samples from those receiving control feed. The upregulation of *SelenoW* and *SelenoH* as well as the downregulation of the genes *gpx3*, *sod1*, *ifnɤ* and *cox2*, might reflect a better antioxidant defence in muscle tissue from Se-supplemented pigs as products of all these genes are associated with the antioxidant system.

Prior to LPS application, the expression of three selenogenes (*Seleno N*, *SelenoS*, *thioredoxin reductase 1* [*txnrd1*]) and one non-selenogene (*catalase*) was higher in selenite-fed pigs compared with those fed the other diets. Txnrd1 is involved in the selenite metabolism, which can trigger the formation of reactive oxygen species (ROS) causing stress affecting the endoplasmatic reticulum (ER). Both SelN and SelS protect the ER from ROS. Catalase catalyses the decomposition of the ROS hydrogen peroxide. The observed downregulation of *glutathione peroxidase* (gpx) *1* in the same pigs' blood isolated 24h after LPS exposure, might illustrate a re-distribution of Se to higher-ranking selenoproteins, e.g., the gastrointestinal GPx. The gene encoding the anti-inflammatory cytokine interleukin 10 (IL-10) was up-regulated post-LPS in pigs receiving SeMet indicating a stronger depression of the inflammatory response. This may reflect an improved regulation of the immune response channelling more energy to growth and maintenance.

An evident dietary effect was found for Se concentrations in specific organs and tissues (i.e., myocardium, liver, and skeletal muscles) with the highest Se

concentrations in samples from pigs fed SeMet-enriched feed. This is due to the substitution of methionine with SeMet in the amino acid chain of proteins highlighting the fact that Se yeast contains a mixture of Se species compared with pure SeMet sources. In conclusion, the results from this trial indicated a generally better Se status and an improved antioxidant capacity in pigs given organic Se supplements in their feed compared with pigs receiving inorganic Se.

In the sows-offspring trial, sows were fed diets with selenite (0.40 or 0.60 mg Se/kg feed) or SeMet (0.26 or 0.43 mg Se/kg feed) supplements beginning approximately one month before farrowing and up until the end of the lactation period (**Paper III**). Sows fed SeMet-supplemented diets showed higher feed intake and higher levels of total Se, SelP, SeAlb, and SeMet in their colostrum samples. In milk samples collected from these sows at weaning, total Se and SeMet levels remained higher than in those given selenite. In conclusion, colostrum and milk from SeMet-fed sows were better Se sources for the offspring than colostrum and milk from sows fed selenite-enriched feed. An improved Se status in the offspring would strengthen the offspring's antioxidant capacity and thereby increase the average daily weight gain.

In the study of offspring (**Paper IV**) from the above-mentioned sows, neonatal piglets from sows fed SeMet-0.43 had lower red blood cell counts, haemoglobin, and haematocrit compared with the group NaSe-0.40 (standard selenite level). The haematological findings in the SeMet-fed groups are consistent with a hepatic transselenation (TS) pathway still immature in pigs at birth, thus leaving Se bound in Se species inaccessible for selenoprotein synthesis. Hence, the supplementation of maternal diets with selenite at EU-approved dietary levels (NaSe-0.40) seemed to be favourable for prenatal erythropoiesis.

Piglets originating from sows fed SeMet-supplemented feed were born with higher plasma levels of GPx3, an enzyme synthesized in the kidneys and

known to protect against oxidative stress, and, had a higher estimated Se intake via colostrum (**Paper IV**). Also, these piglets showed higher body weights from 24 days of age compared with the NaSe-0.40 group. In 38-days-old piglets from NaSe-0.60 fed sows, enzymatic activities of aspartate transaminase and lactate dehydrogenase were higher, possibly indicating damage to cells with high metabolic activity due to oxidative stress. Se concentrations in the myocardium, diaphragm, and *M. semitendinosus* of neonatal piglets were highest in those originating from sows fed SeMet-supplemented feed. Higher GPx3 plasma levels in perinatal pigs, especially from SeMet-supplemented sows, would probably increase protection against birth-related oxidative stress. Tissue Se concentrations showed that SeMet was transferred more efficiently than selenite via the placenta.

Taken together, the hepatic TS-pathway is immature in neonatal piglets and does not provide enough Se for optimal synthesis of selenoproteins. Thus, the inorganic Se source selenite, directly accessible for the synthesis of selenoproteins, is of special importance for neonatal piglets. Unlike inorganic Se, SeMet can be stored in non-selenoproteins, and thus stabilise the Se metabolism over time and assures a higher transfer of Se to porcine offspring and non-vegetarian humans. In addition, results on Se speciation showed that Se distribution depended on the pig's age in plasma (**Paper IV**) and on its physiological stage in colostrum and milk (**Paper III**). The Se concentration in porcine kidney and liver seemed to be age-dependent, too.

Based on the results from this project, a combination of the Se sources SeMet and selenite (0.2 and 0.25 mg/kg, respectively) in complete feed for pigs, still complying with the EU regulations, is recommended. This might be beneficial in diets for breeding sows in terms of both fertility parameters and the offspring's growth performance, the robustness of their antioxidant system and thereby their feed efficiency. However, more research seems to be important to further elucidate the actual Se requirements in high-yielding pigs.

Sammendrag (Summary in Norwegian)

Selen (Se) er et sporelement som er essensielt for pattedyrenes helse, men er toksisk i høyere konsentrasjoner. Sporelementets biologiske rolle i levende organismer er knyttet til aminosyren selenocystein (SeCys). Som del av SeCys i selenoproteiner transporteres Se gjennom kroppen og selenoenzymer har en høyere reaktivitet sammenliknet med Cys-analoger.

Korn er den naturlige hovedkilden til Se i kommersielt svinefôr. Jordsmonnet i Norges innland inneholder vanligvis lite Se, og dette medfører lave Se-konsentrasjoner i kornet som dyrkes. Siden 1980 har Se blitt tilsatt norsk grisefôr i form av den uorganiske Se-kilden natriumselenitt (selenitt).

Til tross for at Se har blitt tilsatt kommersielt svinefôr i konsentrasjoner opp mot den øvre tillatte grenseverdien (0.5 mg Se/kg fôr) i henhold til EUs regelverk, har man funnet sykdomstilfeller relatert til Se-mangel i norske svinebesetninger. Den norske fôrindustrien har forsøkt å forebygge dette ved å tilsette vitamin E (vitE) i høyere konsentrasjoner - fordi mengden som kan tilsettes ikke er begrenset i regelverket. Både Se, i selenspecies (Se-spesies), og vitE har antioksidative egenskaper, og mangel ytrer seg i problemer relatert til oksidativ stress. Se og VitE antas å ha en innbyrdes relasjon i forebyggingen av forskjellige sykdommer hos dyr og mennesker. Tilførsel av den ene kan redusere symptomer forårsaket av mangel på den andre.

Prosjektets hovedmål var å sammenligne effektene av L-selenometionin (SeMet) med selenitt i svinefôr på (a) hurtigvoksende og fôreffektive grisers helse hvis tilsatt opp mot største i EU-tillatte Se-nivå i fôret, (b) Se-opptak i plasma, kolostrum og melk samt i vev hos griser i ulike aldersgrupper, og (c) innflytelsen av Se-kilde og Se-nivå i fôret på ekspresjon av selen- og ikke-selengener, samt forskjellige kliniske parametere.

Det ble gjennomført en undersøkelse av akutt hjertedød (Mulberry heart disease, MHD; **Publikasjon** (Publ.) **I**) som deskriptiv studie, hvor Se-

konsentrasjoner ble målt i organer og vevsprøver fra griser med MHD. Grisene hadde blitt funnet død uten forutgående tegn på sykdom og viste forandringer forenlig med MHD til tross for at de fikk tildelt fôr som var tilsatt Se og vitE etter gjeldende EU regelverk og aktuelle anbefalinger.

Videre ble to fôringsforsøk som inkluderte 4 dietter hver gjennomført for å nå de ovennevnte målene. En ren slaktegrisstudie (**Publ. II**) inkluderte 24 avvente slaktegriser. Det neste forsøket med 32 purker (**Publ. III**) og deres avkom (**Publ. IV**) dekket sen-drektighet, grising, laktasjon og avkommenes liv fram til slakt.

Ekspresjon av utvalgte gener ble målt for å undersøke effekter av Se-kilde eller Se-nivå (**Publ. II**). Her ble det undersøkt 1) helblod som ble isolert før og etter en intravenøs injeksjon med lipopolysakkarider (LPS) og 2) prøver fra den store ryggmuskelen (*M. long. dorsi*) som ble tatt ut tre ganger gjennom hele slaktegrisfasen. I tillegg ble det gjennomført kliniske observasjoner gjennom LPS-studiene og måling av totale Se-nivåer i blodplasma, kolostrum, melk og forskjellige vev (**Publ. I-IV**). Den biokjemiske aktiviteten av Se er avhengig av hvilke Se-forbindelser som tas opp, akkumuleres og produseres. Derfor er målinger av Se-spesies (Se-spesiering) essensielt for å forklare både prosesser og eventuelle effektforskjeller observert i purker og deres avkom fôret med dietter tilsatt selenitt eller SeMet i forskjellige konsentrasjoner. Se-spesies ble bestemt i heparinplasmaprøver fra purker og deres avkom i tillegg til kolostrum og melk fra purkene (**Publ. III og IV**).

Feltkasus diagnostisert med MHD ble undersøkt og gjennomsnittlige Se-konsentrasjoner var signifikant lavere i MHD- enn i kontrollkasus i prøver fra hjertemuskelen, ni av ti undersøkte skjelettmuskler, magesekk, duodenum, jejunum, blindtarm, lever, nyre, tymus og hud (**Publ. I**). Resultatene viser at prøver fra skjelettmuskulatur bør inkluderes ved kjemisk analyse i forbindelse med diagnostisering av MHD.

I slaktegrisstudien (**Publ. II**) ble det funnet at selenogenene *SelenoW* og *SelenoH*, som begge koder for thioredoxin-liknende selenoproteiner, var sterkere uttrykt i *M. long. dorsi* hos dyr som fikk Se i fôr (0.3 mg/kg) enten i form av selenitt [NaSe], selengjær [Se yeast; Se gjær] eller SeMet over en periode på 64 dager, sammenlignet med kontrolldyr som fikk fôr uten tilsatt Se. I *M. long. dorsi* fra griser som fikk fôr med Se tilsetning, ble genene *gpx3* og *superoxide dismutase 1* (*sod1*; dag 38), *interferon-gamma* (*ifnγ*) and *cyclooxygenase 2* (*cox2*; dag 64) uttrykt lavere sammenlignet med griser som fikk fôr uten Se. Oppreguleringen av *SelenoW* og *SelenoH,* samt nedreguleringen av *gpx3* og *sod1*, *ifnγ* og *cox2* gjenspeiler muligens et bedre antioksidativt forsvar hos griser som får Se-beriket fôr, siden produktene av alle disse gener er viktige for det antioksidative systemet.

Før LPS-applikasjonen 45 dager etter forsøksstart viste selenogenene *SelenoN*, *SelenoS* (begge koder for selenoproteiner i endplasmatisk retikulum, ER) og *thioredoxin reductase 1* (*Txnrd1*; koder for en oksidoreduktase) og det selen-uavhengige genet som koder for *katalase*, en viktig antioksidant, et høyere genuttrykk i blod fra griser som ble gitt fôr tilsatt selenitt, enn i de andre forsøksgruppene (**Publ. II**). Txnrd1 er involvert i selenitt-metabolismen, som kan øke oksidativt stress for ER pga. produksjon av frie radikaler. SelN og SelS beskytter ER mot effekter fra frie radikaler. Enzymet katalase avgifter det fri radikal hydrogen peroksid.

I blodet til de samme grisene, 24 timer etter LPS-injeksjon var genuttrykket av *glutationperoksidase (gpx) 1* mer redusert i selenitt-fôret griser enn i griser som fikk fôr tilsatt organisk Se. GPx1 er en annen viktig antioksidant og dens nedregulering kan indikere en omfordeling av Se til viktigere selenoproteiner. I tillegg ble genet *il-10*, som koder for et antiinflammatorisk cytokin, oppregulert i blodet fra griser fôret med SeMet-beriket fôr. Dette indikerer muligens et bedre koordinert immunsvar og derfor en bedre utnyttelse av energi for vekst og vedlikehold hos disse dyrene.

Griser som fikk fôr tilsatt SeMet, hadde høyest Se-konsentrasjoner i organer og vevsprøver som hjertemuskel, lever, og skjelettmuskler sammenliknet med griser fôret med de andre forsøksdiettene. Dette kan forklares med at metionin erstattes med SeMet i kroppens proteiner. Dette viser at Se-gjær inneholder en blanding av Se-species, dominert av SeMet, kontra den rene SeMet-kilden. Det kan konkluderes at resultatene fra dette forsøket indikerer en bedre Se-status generelt, og en høyere antioksidativ status hos griser som får organisk Se via fôret.

I purke-avkom forsøket fikk purker dietter tilsatt selenitt (0.40 og 0.60 mg Se/kg fôr; NaSe) eller SeMet (0.26 og 0.43 mg Se/kg fôr) fra ca. en måned før forventet grising fram til avvenning (**Publ. III**). Purker som fikk fôr tilsatt SeMet, hadde høyere fôropptak og høyere nivå av totalt Se, SelP, SeAlb og SeMet i kolostrum. I melkeprøver (tatt ved avvenning) fra de samme purkene var totalt Se og SeMet fortsatt høyere. Man kan konkludere med at kolostrum og melk fra purker som fikk tilført SeMet via fôret, sannsynligvis er bedre Se-kilder for avkommene enn kolostrum og melk fra purkene som fikk selenitt-beriket fôr. En bedre Se-status hos avkommene vil forbedre deres antioksidative kapasitet og dermed kunne øke deres daglige tilvekst.

I undersøkelsene av avkom (**Publ. IV**) fra disse purkene, inneholdt blod fra nyfødte griser fra purker fôret med NaSe-0.40 (vanlig nivå av selenitt tilsatt svinefôr) et høyere antall erytrocytter, høyere hematokrit og mer hemoglobin enn blodet fra purker fôret med SeMet-0.43. De hematologiske funnene er forenlige med en fortsatt umoden hepatisk «TS-pathway» ved fødsel, som begrenser tilgjengelighet av Se som er bundet i Se-species for selenprotein-syntese. Resultatene tyder på at selenitt-tilsetning til purkenes fôr gjeldende grenseverdi på 0.5 mg/ kg, kan være gunstig for prenatal erytropoese.

Avkom etter purker som hadde fått fôr tilsatt SeMet, ble født med høyere plasma GPx3 nivå (**Publ. IV**), et enzym som syntetiseres i nyrene og har antioksidativ virkning. Deres estimerte Se-inntak via kolostrum var også

høyere. I tillegg viste disse avkommene en bedre vektutvikling fra dag 24 sammenlignet med avkom etter purker som hadde fått NaSe-0.40-dietten. Høyere plasma GPx3 nivåer hos perinatale griser, spesielt hvis purkene fikk SeMet beriket fôr, vil sannsynligvis øke de nyfødtes motstandsdyktighet mot oksidativt stress i forbindelse med grisingen.

Ved 38-dagers alder var enzymaktiviteten til aspartat transaminase og laktatdehydrogenase høyere i avkom fra purker fôret med NaSe-0.60-dietten. Dette kan indikere celleskader pga. oksidativt stress. Se-konsentrasjoner i myokard, diafragma og *M. semitendinosus* fra nyfødte spedgriser var høyest når purkene hadde fått fôr tilsatt SeMet.

Den hepatiske TS-pathway er umoden hos nyfødte spedgriser og gir ikke nok Se for optimal syntese av selenoproteiner. Dermed synes selenitt som er direkte tilgjengelig, å være en viktig Se-kilde for nyfødte griser. Samtidig kan lagring av SeMet i non-selenoproteiner stabilisere Se-metabolismen over tid og sikre høy overførsel av Se til avkom. I tillegg viste Se spesieringsresultater at fordelingen av Se er avhengig av grisens alder i plasma, mens den avhenger av grisens fysiologiske stadie i kolostrum og melk. Også Se konsentrasjonen i nyre og lever virket til å avhenge av grisens alder.

Når man vurderer fordelene med begge Se kildene brukt i disse fôringsforsøkene, synes en kombinasjon av selenitt og SeMet (hver opp til 0.2 – 0.25 mg Se/kg) i fullfôr til gris å være den beste løsningen. Dette er innenfor EUs gjeldende grenseverdi på 0.5 mg Se/kg, og kan være fordelaktig med tanke på purkenes reproduksjon og avkommenes vekstpotensiale, immunsystemets motstandsdyktighet og dermed fôrutnyttelse. Basert på prosjektets begrensninger konkluderes det med at mer forskning er nødvendig for å kartlegge selenbehov hos modern, hurtigvoksende griser med stor muskelmasse.

List of papers

I. M. Oropeza-Moe, **M. Falk**, M. Vollset, H. Wisløff, A. Bernhoft, T. Framstad and B. Salbu
A descriptive report of the selenium distribution in tissues from pigs with mulberry heart disease (MHD). Porc Health Manag 5, 17 (2019)

II. **M. Falk**, A. Bernhoft, T. Framstad, B. Salbu, H. Wisløff, T. M. Kortner, A. B. Kristoffersen, M. Oropeza-Moe
Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs. JTEMB Volume 50, March 2018, Pages 527-536

III. **M. Falk**, P. Lebed, A. Bernhoft, T. Framstad, A. B. Kristoffersen, B. Salbu, M. Oropeza-Moe
Effects of sodium selenite and L-selenomethionine on feed intake, clinically relevant blood parameters and selenium species in plasma, colostrum, and milk from high-yielding sows. JTEMB Volume 52, December 2019, Pages 176-185

IV. **M. Falk**, A. Bernhoft, E. Reinoso-Maset, B. Salbu, T. Framstad, H. Fuhrmann, M. Oropeza-Moe
Beneficial antioxidant status of piglets from sows fed selenomethionine compared with piglets from sows fed sodium selenite. JTEMB Volume 58, December 2020, 126439

Introduction

Modern swine production with a focus on productivity, leaner pork, and feed efficiency

Since the 1970s and onwards, the geno- and phenotype of pigs has undergone radical changes reflected in high productivity such as faster growth, higher lean body mass and reduced backfat, improved feed efficiency, and longevity in the breeding herds [1, 2]. Systematic breeding programmes have developed the Norwegian Landrace and Norwegian Duroc into a lean and feed-efficient breed [3].

Since the 1990s, genetic progress in sows' fertility traits has been evident especially in litter size at birth, litter weight at weaning and vitality of piglets [4-6]. As an example, selection for litter weight at weaning in maternal lines from 2004 to 2012 resulted in TN70-sows, based on the Norwegian Landrace (Norsvin) and the Dutch Z-line of Yorkshire pigs, displaying significantly increased milking ability and improvement of other maternal traits [7, 8]. Subsequently, the body weight (BW) of 3-weeks-old piglets increased by 700 g on average [8]. However, the maintenance requirements of today's modern genotypes are higher since the need for energy to maintain lean tissue is higher compared to lipid tissue [5, 9]. Additionally, the reduction of body reserves, e.g., backfat, combined with the high growth potential makes young and lean sows more prone to nutritional stress, especially during lactation, compared with sows with more body reserves [10, 11]. In addition, it was hypothesized that the higher lean body mass, increased litter size, and faster litter growth have increased the requirements for essential amino acids in sows, e.g. lysine, threonine, valine [5] and methionine + cysteine [12].

Regarding grower-finisher pigs, breeding goals focusing on improvements of average daily weight gain (ADG) or days to market to provide meat at a lower price [4, 13] significantly reduced the rearing period of finishing pigs [14]. The cause for the increased growth rate in pigs is the extraordinary increase

in muscle tissue growth per kg feed (feed efficiency). After comparing the growth potentials of Danish Landrace pigs from 1976 and 1995 it was suggested that increased muscle fibre number and DNA deposition (satellite cell proliferation) may have contributed to the enhanced muscle growth [15].

In pigs, a high genetic growth capacity appears to be closely related to high feed intake during the growth period at days 95 to 140 [16]. However, average daily feed intake is considered to be negatively correlated to the leanness of the meat; selection for increased leanness may therefore lead to a decrease in the feed intake capacity of pigs [16]. An animal's efficiency is measured by converting feed mass into increased body mass. The feed conversion ratio, and thus the feed efficiency, have improved for all production animal species [17].

Challenges related to production traits of modern pigs

Selection for productivity increases physiological demands [18] and might increase stress and disease susceptibility. It has been stated that modern breeding techniques can affect animal welfare; a rapid modulation of certain genetic traits can easily result in an insufficient adaptation to the increased performance of organs and/or the skeleton [13]. Highly productive pigs may therefore have difficulties coping with environmental challenges and may be at risk of acquiring physiological, and immunological problems [18]. Taken together, modern pigs may become less resilient [19].

High growth rates in fast-growing, lean pigs might augment the oxygen demand at the tissue level to sustain metabolic needs, as shown in broiler chickens [20]. In addition, pig husbandry in the EU and in Norway in particular has very high standards (see Table 1), such as minimum requirements for housing and feeding [21, 22]. Also, the incidence of viral and bacterial diseases is low in the Norwegian swine population [23-25].

Table 1 Differences between Norwegian and European legislation concerning pig production

EC 2001/93/EC [21]	Norwegian regulations [22]
Noise	
Constant noise >85 dB shall be avoided, as shall constant or sudden noise.	Constant noise >65 dB shall be avoided.
Light	
>40 LUX for min 8 hours (h) per day.	>75 LUX for min 8 h per day.
Accommodation	
Physically and thermally comfortable as well as adequately drained and clean, which allows all the animals to lie down at the same time.	Physically and thermally comfortable as well as adequately drained and clean, which allows all the animals to lie at the same time, and the flooring in the laying area shall be solid, deep-straw or dry litter.
Floors	
Must be smooth and not slippery and prevent injury and suffering. They must be suitable for the size and weight of the pig, and if no litter is provided, form a rigid, even and stable surface.	Must be smooth and not slippery and prevent injury and suffering. They must be suitable for the size and weight of the pig, and if no litter is provided, form a rigid, even and stable surface. Solid, deep-straw or dry litter in laying area.
Feeding	
All pigs must be fed at least once a day. If fed in groups and not as lib or by individual automatic system feeding, then each pig must have access to the feed at the same time.	All pigs must be fed at least once a day. Pigs before puberty or during lactation must be fed at least twice/day. If fed in groups and not as lib or by individual automatic system feeding, then each pig must have access to the feed at the same time. Sows, gilts and boars must have access to roughage (hay, straw or other high fibre diet) that can provide satiety in addition to chewing.

Table 1 (contd.) Differences between Norwegian and European legislation concerning pig production

EC 2001/93/EC [21]	Norwegian regulations [22]
Water	
All pigs >2 weeks of age must have permanent access to a sufficient quantity of fresh water.	All pigs must have permanent access to a sufficient quantity of fresh water of adequate chemical and bacteriological quality.
Procedures	
All procedures carried out for other than therapeutic or diagnostic purposes or for the identification of pigs and resulting in damage to or the loss of a sensitive part of the body or the alteration of the bone structure shall be prohibited, with the following exceptions: • A uniform reduction of corner teeth by grinding or clipping days of age (or boar tusks). • Docking of a part of the tail. • Castration of male pigs by other means than tearing of • Nose ringing in outdoor accordance with national legislation. Teeth clipping nor tail docking routinely without evidence of damage (only after other measures have been tried). Any of the procedures above by a vet or trained person with appropriate means under hygienic conditions. Castration/docking >7th day of life only by vet under anaesthetic and prolonged analgesia.	All procedures carried out for other than therapeutic or diagnostic purposes or for the identification of pigs and resulting in damage to or the loss of a sensitive part of the body or the alteration of the bone structure shall be prohibited, with the following exceptions: • A uniform reduction of corner teeth by grinding <7 days age (or boar tusks). • Castration of male pigs by other means than tearing of tissues <28 days old. • Tail docking on veterinary indication. • Castration/docking only by a veterinarian under local anaesthetic and prolonged analgesia.

The sum of these factors might enable the pigs to express their full genetic potential for fast and efficient lean growth, high ADG, feed efficiency, and high prolificacy. Unfortunately, this optimization probably increases the risk of a ROS-antioxidant-imbalance, also called oxidative stress, in modern pig hybrids. Thus, in theory, the modern fast-growing, leaner and more feed-efficient pig has a greater requirement for nutrients with antioxidant properties including Se and vitamin E (vitE) than did the pig hybrids used in the 1980s. However, the combination of high growth rate with high feed efficiency is equivalent to less feed, thus fewer nutrients including Se, for growth and maintenance of more muscle mass [19]. Subsequently, suboptimal intake of exogenous antioxidants can lead to nutritional deficiency [19].

Consequently, a precisely balanced diet is imperative to balance the effects of feed efficiency and growth capacity with the pig's ability to cope with stress [26]. Regarding the dietary Se concentration in pig feed, an increase has been impossible due to the limitation of Se to a maximum of 0.5 mg/kg full feed by the EU legislation [27]. The limit is based on the narrow range between Se requirements and toxic doses of Se, which depends on its chemical form and the route of exposure [28].

This raises the question as to whether today's feeding strategies meet the Se requirements in high-yielding pigs [29, 30].

Oxidative stress and antioxidant defence

Inhaled oxygen is mostly "reduced directly to water without ROS release" [31]. A small fraction "of the oxygen consumed is reduced resulting in conversion of molecular oxygen (O2) to superoxide anion radical (O2•−) followed by reduction to hydrogen peroxide" (H2O2) [31]. Thus, free radicals are produced in the body due to metabolism (oxidation), due to a series of stressors (e.g., contaminants, radiation) and due to the Fenton reaction (reaction between e.g., divalent Fe and H2O2). Following induction of free

radicals (e.g., splitting of water molecules into H• and hydroxyl anion OH• radicals), recombination and interactions would result in e.g., H2O2 and a series of reactive oxygen species (ROS) within cells. Then, antioxidants play an important role to detoxify ROS to avoid negative effects. Disturbance of the ROS-antioxidant-balance might be caused by exposure to stressors (e.g. increased levels of endogenous and exogenous pro-oxidants) as well as depletion of antioxidant reserves or decrease in production of antioxidants [31]. When production of ROS overloads the antioxidant defences, ROS can lead to a variety of physiological and biochemical changes that could induce damage to biomolecules such as DNA and RNA, proteins, lipids, etc., as well as causing damage to cell membranes and ultimately cell death [32]. There is growing evidence that oxidative stress is involved in the pathogenesis of various disorders and diseases, which draws attention to antioxidants for prevention as well as for treatment of such conditions.

A series of molecules, known as antioxidants, such as Se-containing enzymes, vitE, thiols, or ascorbic acid (vitamin C, vitC) interact with different ROS resulting in detoxifying effects. Antioxidants can be grouped into exogenous (dietary) and endogenous antioxidants, or non-enzymatic and enzymatic antioxidants [33]. Enzymatic (endogenous) antioxidants include selenoenzymes like e.g., glutathione peroxidases (GPx) as well as the non-selenoenzymes superoxide dismutase (SOD), catalase, thioredoxin, and glutathione-S-transferase (GST), commonly requiring NADPH as a reducing equivalent. Non-enzymatic antioxidants include low-molecular-weight compounds such as vitC (species-dependent, if endogenous or not), vitE (exogenous), and glutathione (GSH; endogenous). To prevent cellular damages associated with oxidative stress it is important to balance the ratio of antioxidants to oxidants by supplementation or by cellular induction of antioxidants [34]. Requirements of an individual antioxidant are to some extent dependent on the presence of others [35] as described in the case of Se and vitE [36-38]. The cell membrane-associated antioxidant vitE is

interrelated with the mainly intracellular acting Se [39], contributing to faster cellular regeneration.

In the present project, the focus was mainly on the properties of Se, and, to some extent, on vitE, both of which are available as dietary supplements.

Stressful periods for pigs at different ages

Pigs are full-grown at about 3–3.5 years of age [40]. Only boars and sows can reach the full-grown stage in modern pig production. Thus, all stages in modern pig production include growing pigs.

Throughout a pig's life, several events can cause an increase in oxidative stress. Sows are exposed to different kinds of stress such as social stress, heat stress, weaning stress, possibly causing oxidative stress during their reproductive cycle [41-46]. Enhanced maternal oxidative stress and subsequently foetal exposure to excess glucocorticoid levels can negatively influence offspring physiology, such as birth weight, glucose homeostasis, and the hypothalamic-pituitary-adrenal axis activity [47].

Birth exposes the neonate to the much higher extrauterine oxygen levels (appr. 100 mmHg oxygen tension (PO2) versus intrauterine hypoxic 20–25 mmHg PO2) [48]. This increase in oxygen tension induces an elevated production of ROS, detectable in the blood of neonatal piglets and oxidative stress may develop in the neonate, also given its naïve antioxidant system [49] with low levels of antioxidants such as Se and vitE [48, 50]. On the other side, the oxidative stress during the foetal-to-neonatal transition enhances the antioxidant defence and pulmonary surfactant maturation [51].

Postnatal, the antioxidant properties of colostrum are of special importance both to the neonates' health and the quality of the subsequently synthesized milk [52]. The treatment of piglets with iron during the first days of life delivers excess iron, which is potentially harmful due to the formation of toxic ROS via the Fenton reaction [53]. Iron donates or accepts free electrons via intracellular reactions and supports the production of free radicals (here

OH•). The Fenton reaction describes the formation of ROS by a reaction between transition metal ions such as iron (II) (Fe^{2+}) and H_2O_2 [54].

Also weaning is a challenging period for piglets due to nutritional, intestinal, environmental, and social changes at an age where oxidative products increase in the plasma [55-58]. In Norway the average weaning age is 33.3 d (2011-2020) [59, 60].

Selenium

In humans and other mammals, Se status as well as the incidence of diseases and reproductive problems related to oxidative stress, i.e., infertility and lethargic neonates, are inversely correlated [61-64].

After the discovery of Se in 1817 by Jöns Jacob Berzelius, it was deemed toxic for more than one hundred years. Research on bacteria of the family Enterobacteriaceae and liver necrosis in rats revealed this element to be an essential micronutrient for both prokaryotes [65] and eukaryotes [66]. Se enters the food chain through plants and marine products, and several factors determine its concentration in feed and foods [67-73]. A selection of factors is presented in Table 2. The herbal Se concentration also depends on the plants' ability to absorb and accumulate this element and on the soil's mineral structure [74, 75].

In the mammalian body, Se mainly occurs as a substitute for sulphur in the amino acids cysteine and methionine forming SeCys and SeMet. A few dietary factors are known to affect the uptake of Se in the organism [76]. Dietary proteins can enhance the uptake of Se from SeMet [77] due to replacement of SeMet by methionine in the amino acid chains of non-seleno body proteins. Thus, methionine promotes SeMet's entrance into the transselenation (TS-) pathway, making its Se accessible for the SeCys synthesis (see Figure 1) [78, 79]. Also, vitA at high levels, vitC, vitE, and other antioxidants appear as enhancers of the uptake of dietary Se in humans and animals via various mechanisms [77, 80].

However, several metals and high dietary sulphur (S) may decrease Se uptake in animals [77, 81], as many Se compounds are absorbed by the same mechanism as their S analogues [76].

Table 2 Geological and geographical factors probably influencing Se concentration in feed and food [based on 76]

Geological and geographical factors of Se	Property
Soil	Differs in selenospecies (Se species) and Se concentration
pH	Determines to some extent the nature of the Se species and influences its bioavailability to plants
Amounts of organic matter, iron hydroxides, Al compounds and clay	Can bind Se, thus reducing its bioavailability to plants
Amounts of S-species (e. g. from S fertilizers)	Can compete with Se for absorption
Rainfall	Can leach Se out of the soil
Soil microbes	Can convert insoluble forms of Se to soluble forms

Absorption of Se is neither homeostatically regulated nor believed to be affected by nutritional status [76, 82]. The absorption, metabolism, distribution within the body of humans and animals, and excretion of Se depend on several factors, particularly its physico-chemical form as well as its total dietary concentration [77].

Different physico-chemical forms of a particular element or its compounds are referred to as “species” [83]. In the case of Se, these species include inorganic Se salts, selenized amino acids, selenoproteins, selenized proteins like selenoalbumin (SeAlb), selenosugars and other organic Se compounds. Absorption of organic Se is generally high; about 80% from food [84]. Clausen and Nielsen [85] suggested that organic Se is more bioavailable for humans than the inorganic forms selenate and selenite, and showed that whole-blood-Se levels continued to rise longer in those receiving organic Se instead of inorganic Se. Furthermore, organic Se showed lower systemic toxicity,

greater resistance to chemical changes, higher stability during food processing, and relatively low clearance rates than inorganic Se [86].

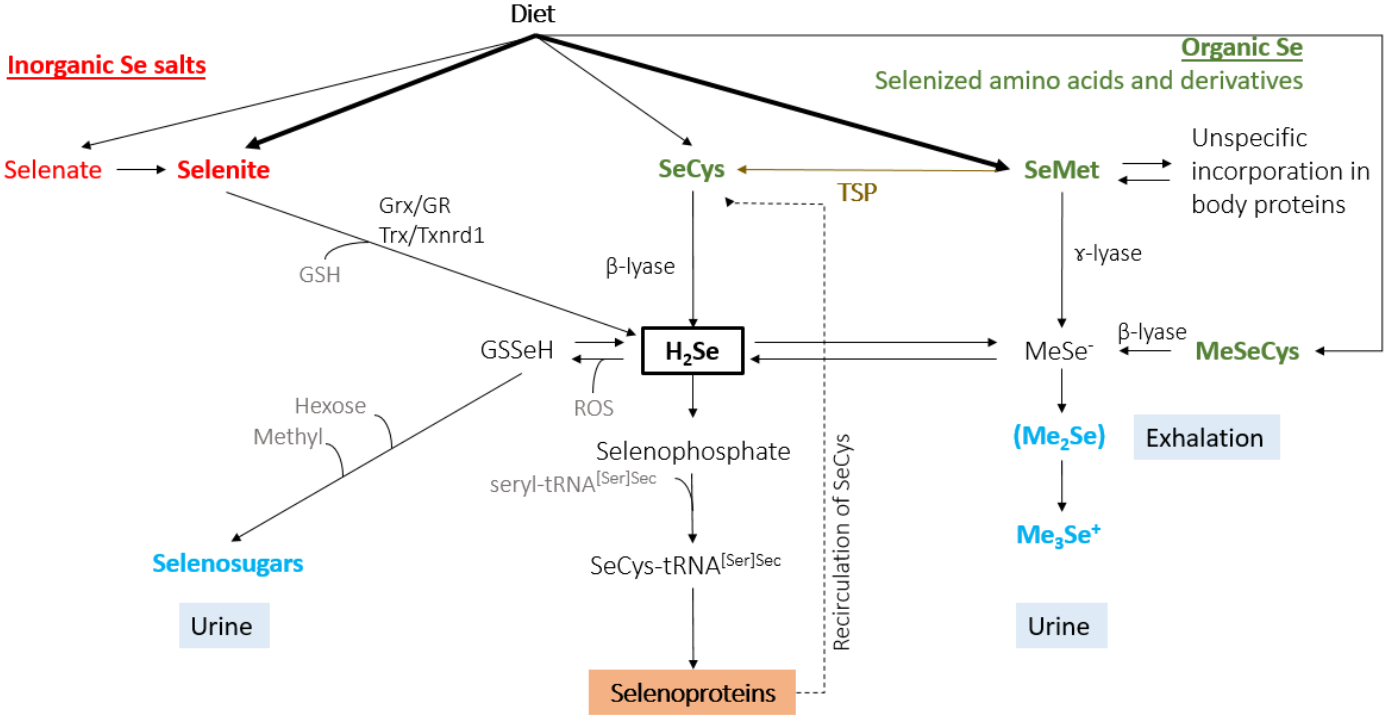

Figure 1 Schematic presentation of the metabolism of dietary selenium *(Se; red – inorganic; green - organic): The reduction of selenite requires glutathione (GSH), glutaredoxin (Grx), glutathione reductase (GSR) and/or thioredoxin (Trx)/thioredoxin reductase 1 (Txnrd1). SeCys is specifically incorporated into selenoproteins (orange) via selenide (H_2Se) and synthesis of selenophosphate. Selenocysteine (SeCys) from selenoproteins is degraded and reused. Selenomethionine (SeMet) from body proteins can split off*

methylselenide or be converted to SeCys via the transselenation pathway (TSP). SeMet may also unspecifically replace its sulphur analogue methionine in proteins. Among excretory metabolites (blue) are selenosugars and at high doses trimethylselenonium ion (Me_3Se^+). Intoxications may lead to excess dimethylselenide (Me_2Se) that may cause garlic breath. Surplus selenide may redox cycle and produce reactive oxygen species (ROS). Adapted from Alexander et al. [87].

Selenoproteins

Se is essential for the synthesis of selenoproteins (Table 3) and is an indispensable component of the enzyme that catalyses the binding of SeCys to the amino acid chain of selenoproteins [78, 79]. Se is covalently attached in a SeCys residue, which is co-translationally inserted into the polypeptide chain of selenoproteins [88-90]. As a superior catalyst, SeCys supports a higher catalytic efficiency than cysteine alone owing to, amongst other factors, its higher polarizability and lower pKa-value (~5.2) [91].

Se from both SeMet and the oxidized inorganic forms (selenate, selenite) can undergo reductive metabolism yielding selenide (H_2Se), the basis for the synthesis of selenoproteins [92] (Figure 1). H_2Se can be converted to selenophosphate ($HSePO_3^{2-}$) by selenophosphate synthetase [76]. Oxidation of excess H_2Se leads to the production of superoxide ($^{\bullet}O_2^-$) and other ROS [92]. The selenoenzyme Txnrd1 is implicated in the reductive assimilation of selenite by generating selenide for selenoprotein synthesis [93], takes part in the regulation of the redox state of cells [94] and reduces several low-molecular-mass compounds such as oxidized glutathione (GSSG), selenodiglutathione (GSSeSG) and H_2O_2 [95]. GSSeSG is generated in erythrocytes during the selenite metabolism [96].

Until now, 25 selenoproteins have been described in humans [97], and all of them have been studied in pigs (see Table 3). Cells produce selenoproteins based on the properties of their tissue and the importance of the respective selenoprotein (Se hierarchy) [98, 99]. Among the selenoproteins, selenoprotein P (SelP) is unique due to its multiple SeCys residues and its function as a Se transport protein. SelP and its receptors regulate Se uptake in a tissue-specific manner [100-103] and depending on the Se status.

Low levels of SelP cause a rapid drop of the concentrations of GPx1, GPx3, and other low ranking selenoproteins, preserving Se for the synthesis of “essential” selenoenzymes [104].

Table 3 Selenoproteins in humans and pigs

Abb.	Tissue	Loc.*	Name and Function ***	AO**
Family of deiodinases				
Dio1^	Thyroid, liver, kidney, etc.	I	Iodothyronine deiodinase 1; transmembranous; production of triiodothyronine (T_3) in the thyroid and peripheral tissues; can deiodinate both rings of the thyroxine (T_4) or T_3 molecules; activates T_4 to produce T_3 and inactivates T_4 [105, 106]	
Dio2^	Thyroid, brain, pituitary, muscle, BAT, ear, heart, etc.	I	Iodothyronine deiodinase 2; integral component of the membrane; ER^^; T_3 production in peripheral tissues [105, 106]	
Dio3^	Pregnant uterus, placenta, foetal/neonatal tissues	I	Iodothyronine deiodinase 3; membrane protein; important in homeostatic regulation in maintaining T_3 levels at the plasma and cellular levels; can only deiodinate the inner ring of the T_4 or T_3 molecules and is the major inactivating enzyme; prevents T_4 activation and inactivates T_3 [106]	
Family of glutathione peroxidases: majority with a tetrameric structure with one Se atom in each subunit				
GPx1	Ubiquitously expressed	I	Cellular/classical GPx; one of the most abundant members of the GPx family of enzymes; reductive detoxification of peroxides; depends on GSH as obligate co-substrate; counteracts oxidative attacks; is lower ranked in the hierarchy of GPx [107]	X
GPx2	Predominantly in intestine	I	Epithelial-specific enzyme; anti-apoptotic function in colon crypts; helps to maintain intestinal mucosal integrity [108]	X
GPx3	Widely expressed, mainly produced by kidney	E, S	Plasma-GPx; a major antioxidant enzyme in plasma; comprises 10–25% of plasma Se; protects numerous types of epithelia; catalyses the reduction of H_2O_2 and lipid hydroperoxides; maintains the bioavailability of nitric oxide (NO) in the vasculature [109]	X

Table 3 (contd.) Selenoproteins in humans and pigs

Abb.	Tissue	Loc.*	Name and Function ***	AO**
GPx4^, PHGPx	Widely expressed	I	Phospholipid hydroperoxide GPx; membrane-associated; present at high concentrations in the testes, where it is essential for sperm motility and viability; plays a role in the synthesis of prostaglandins; reduces phospholipid hydroperoxides in membranes; acts in conjunction with α-tocopherol (vitE) [110-114]	X
GPx6	Testes, embryonic tissues, olfactory epithelium	E, S	Involved in the arachidonic acid metabolism; odorant metabolizing protein; GPx1 homologue? [90]	X
Redox (rdx) family: thioredoxin-like fold structure and a conserved CxxC or UxxC (C - Cys; U - SeCys); redox function suggested				
SelH	Ubiquitously expressed	I	Essential regulator of redox homeostasis; SelH deficiency induces inflammatory response and activates the p53 pathway; suppresses cellular senescence; significant GPx activity in presence of H_2O_2 [115-117]	X
SelM	Widely expressed	I	ER^^-resident redox protein; its N-terminal signal peptide is necessary for protein translocation [118, 119]	
SelT	Widely expressed	I	In Golgi and ER^^; involved in redox regulation and cell anchorage; regulation of Ca^{2+} homeostasis; loss of SelT elevates expression of SelW [120, 121]	X
SelV	Widely expressed		Might be involved in differentiation, growth, and activation of cells	

Table 3 (contd.) Selenoproteins in humans and pigs

Abb.	Tissue	Loc.*	Name and Function ***	AO**
SelW	Widely expressed		Smallest mammalian selenoprotein; abundant in muscle; reduced levels found in white muscle disease cases; predominantly in the cytoplasm, but a small proportion is associated with the cell membrane [122-124]	X
Sep15, SelF	Widely expressed	I	ER^^-resident redox protein; takes part in the process of correct disulphide bond formation	X
Family of thioredoxin (Trx) reductases/ pyridine nucleotide oxidoreductases: Homodimeric flavoproteins that catalyse the reduction of Trx by NADPH				
Txnrd1	Widely expressed	I	Thioredoxin reductase 1; cytosolic; its carboxyl-terminal SeCys is a cellular redox sensor; redox-regulated cell signalling; reduces thioredoxins and other substrates; important for Se metabolism and protection against OS [125, 126]	X
Txnrd2	Liver, kidney, adrenal gland, heart, testis	I	Thioredoxin reductase 2; mitochondrial; part of the primary line of defence against H_2O_2 produced by the mitochondrial respiratory chain [125, 126]	X
Txnrd3	Widely expressed	I	Thioredoxin reductase 3; maybe a mitochondrial enzyme [126]	X
Unclassified Se-proteins				
SelI	Widely expressed	I	Membrane-bound; mammalian form of ethanolamine (PE) phosphotransferase; involved in production of PE – an abundant phospholipid in mammals [166]	
SelK	Widely expressed	I	ER^^; Evt. regulation of cellular redox balance; stress-regulated protein protecting from ER-stress induced apoptosis; ERAD complex (SelS-protein p97-SelK) important in ER-associated degradation and ER stress; required for Ca^{2+} flux in immune cells; involved in T-cell	X

Table 3 (contd.) Selenoproteins in humans and pigs

Abb.	Tissue	Loc.*	Name and Function ***	AO**
SelK contd.			proliferation, T-cell and neutrophil migration, macrophage uptake of low-density lipoprotein and foam cell formation [127-129]	
SelN^	Ubiquitously expressed	I	Located in the membrane of the ER^^; strongly increased expression and accumulation in mononucleated cells surrounding regenerating skeletal muscle fibres	X
SelO^	Widely expressed	I	Mitochondrial; redox-active [130]	X
SelP	Plasma protein; widely expressed, mainly liver	E	SeCys-rich abundant glycoprotein; storage and transport of Se; rapid turnover in rat → consequence: 25% of amount of whole-body Se throughput daily; functions as a phospholipid hydroperoxide GPx in extracellular fluids; heavy metal chelator function through the formation of nontoxic Se–metal complexes [131]	X
SelS	Widely expressed	I	in ER^^; transmembranous; sensitive marker of ER stress; associated with protein degradation; glucose regulated; implicated in the immune and inflammation pathway; protects the ER against OS [132]	X
SelX, MSRB1, SelR	Widely expressed	I	Methionine-R-sulfoxide reductase B1; in mitochondria; oxidoreductase; plays a role in innate immunity by reducing oxidized actin, leading to actin re-polymerization in macrophages; protein repair; response to OS	X
Sephs2	Ubiquitously expressed	I	Selenophosphate synthetase 2; homologue of eubacterial SelD; crucial role in synthesis of selenoproteins [133, 134]	

Sel – selenoprotein; *Localization: E – extracellular, I – intracellular; S - secreted ; **AO – functions as antioxidant; ***References for information given in columns 2-5; ^ Selenoprotein with high priority for Se supply [105]; ^^ER – endoplasmic reticulum

Vitamin/mineral-dependent proteins required for short-term survival and/or reproduction (i.e., "essential") are predicted to be protected in case of deficiency over other "nonessential" mineral-dependent proteins needed only for long-term health [104]. Five selenoproteins were classified as essential (GPx4, Txnrd1, Txnrd2, Dio3, and SelP) and 7 as nonessential (GPx1-GPx3, Dio1, Dio2, Msrb1, and SelN) [104]. GPx1 and the selenoproteins W, H, and M, responded significantly to Se intake in mice making them biomarker candidates for the Se status [135]. Additionally, SelW has been proposed as a biomarker for the efficacy of Se compounds to act as a source for selenoprotein biosynthesis [136].

Physiological functions of selenium

As an integral part of selenoproteins, Se plays an important role in many physiological processes, such as antioxidant defence, DNA synthesis [137], immunomodulation [138], and prevention of chronic diseases such as some kinds of cancer [139]. It is irreplaceable in the regulation of the immune and reproductive system and supports the cells in heavy metal detoxification [138]. Moreover, this element exhibits antibacterial and antiviral properties and alleviates the course of disease [140-142]. The selenoprotein SelK, for instance, is a transmembrane protein localized to the ER and involved in Ca^{2+} flux in immune cells [143], and SelP functions as an intracellular antioxidant in phagocytes [144]. A declining serum Se status during infections in humans, rodents, and pigs has been observed [145-150], which might be due to a changed pattern of selenoprotein synthesis in the liver and other tissues [148, 151-154]. Sammalkorpi et al. [155] suggested, that the need for Se in intracellular immune reactions stimulated by infection could result in an influx of Se from serum into cells and thereby reduce the serum Se concentration.

Supplementing ruminants with supranutritional levels of Se as Se yeast enhanced mammary gland vascularity [156], improved antioxidant status and

immune responses after calving [157], and improved IgG status of Se-replete calves [158]. Feeding supranutritional Se levels as high-Se wheat grain to lambs increased the nutritive quality of the skeletal muscle [159].

However, excess Se supplementation may harm certain immunological functions [160] and caution should be taken to avoid the toxic effects of Se [161, 162].

Selenium deficiency

Se deficiency occurs in humans and animals living in regions with soils poor in Se [163-165]. Low Se levels are associated with an increased risk of cardiovascular diseases, some types of cancer and might have an adverse impact on the immune system, virulence of pathogens, and fertility [62, 111, 112, 166-174].

The lack of Se influences selenoproteins differently depending on the protein itself and on its localization in the organism [135, 175]. Subsequently, Se deficiency can impair immune and thyroid function, deteriorate viral infections, increasing the risk for nephropathy, neurological disease, infertility or prostate cancer in men [176]. GPx1, for example, has been implicated in the development and prevention of many common and complex diseases, such as cancer and cardiovascular diseases [107].

In addition, decreased expression of selenoproteins may impair biological processes leading to degenerative changes in organs and tissues [177] such as degeneration of hepatocytes and muscle fibres in skeletal muscles or in the myocardium. The sarcoplasmic reticulum of Se-deficient muscles is defective in Ca^{2+} sequestration, resulting in extensive calcification of the muscle tissue [178].

Moderate Se deficiency can be aggravated by simultaneous vitE or C deficiency or by potentially pro-oxidative nutrients such as high amounts of either PUFA or vitC [36-38]**.** The antioxidant activity of one molecule e.g., vitE may evolve into pro-oxidant when the co-antioxidants are exhausted under

conditions of mild oxidation [179] because the corresponding oxidized molecule is not effectively returned to the active form of tocopherol by recycling reactions with other antioxidants [reviewed by 180].

Selenium and vitamin E responsive conditions in pigs

Suboptimal supply of vitE/Se increases morbidity due to deterioration of the immune system and may cause problems during gestation and lactation, thereby negatively influencing the reproduction results [181]. Especially, lower litter size, increased foetal mortality, and weak lethargic neonates have been associated with deficiency of vitE or Se in sows [182].

Se deficiency in pigs leads to degenerative changes in both the myocardium, skeletal muscles, and liver [183, 184]. Despite the low Se concentrations in Norwegian soil [185-188], muscular degeneration in pigs was not referred to as a substantial challenge before the 1950s and 1960s. At that time, pigs received a varied diet based on grounded grains from local mills, including food wastes, and vegetables [13]. After the transition to solely grain-based feed, Se-and-vitE-responsive conditions appeared and were mainly observed in periods of fast growth, especially in pig farms with high ADG rates. Several studies have reported positive correlations between growth rate and levels of oxidative stress in animals [189, 190]. It entails a greater metabolic activity, which generates more ROS, and, additionally, resources are diverted into anabolic pathways and away from repairing oxidative damage to proteins [191].

Mulberry heart disease (MHD), a cardiovascular microangiopathy, resulting in sudden deaths, occurs mostly in piglets after weaning during periods of fast growth, in grower-finisher pigs, and gilts [192, 193]. One possible influencing factor leading to MHD in newly weaned pigs is a sudden reduction in feed intake as MHD pigs often have empty gastric ventricles when necropsied. Individuals with accelerated growth might be more susceptible to starvation during periods of food shortage because their metabolism cannot adapt and down-regulate quickly enough [194, 195]. They might therefore

show a reduced ability to respond to environmental stress [194, 195] which may explain, at least partly, why there are still cases of MHD and nutritional muscular dystrophy (NMD; nutritional myodegeneration, white muscle disease) although the pigs' diets are optimally supplemented with Se and vitE [29, 30, 193, 196]. NMD is a muscular dystrophy caused by a deficiency of Se or vitE or both mainly observed in young, fast-growing animals. In addition, VitE and C levels in blood decrease after weaning [30, 197] possibly reinforcing the imbalance between anti- and pro-oxidants.

Several authors support the concept that cellular damage accumulates as a result of an imbalance between the production of ROS and associated damage and repair [191, 198, 199]. Cellular membranes insufficiently protected by Se or vitE can be damaged by ROS. Subsequently elevated calcium (Ca^{2+}) influx results in "mitochondrial Ca^{2+} overload" and degeneration of muscle fibres as seen in MHD and NMD [200]. Cellular processes that cause cell death may be difficult to elucidate, but there is clear evidence that the pattern of food intake and the slope of the growth curve can influence such processes [191]. A suggested theory has been that Se-and-vitE-responsive conditions in production animals are related to the high growth rates and additionally, at least in pigs, to the high feed efficiency [201].

Furthermore, Mahan and Peters [202] reported a numerically higher percentage of severe splay-leg in neonatal pigs from sows fed a basal, non-supplemented diet or diets fortified with selenite compared with those receiving dietary selenized yeast (Se yeast). Splay-leg describes a truly multifactorial condition with temporarily impaired functionality of the hind leg muscles immediately after birth, resulting in neonatal pigs having a lowered ability to stand and walk.

Selenium requirements

Nutritional recommendations are based on nutritional essentiality to prevent specific deficiency disorders [203] and supranutritional intake or intoxication [76, 78, 204].

Penglase et al. [205] stated that the Se requirement of an animal species is predictable from the SeCys content in the species-specific amino acid chain of the SelP. A variation from seven to eighteen Se atoms in vertebrate SelP, as illustrated in Figure 2, might be a result of Se availability in the original habitats of the respective animal species [205]. Their model, based on this hypothesis, predicts an Se-requirement ranking of vertebrates as followed: mammals < birds < fish (freshwater < marine) [205] (Figure 2).

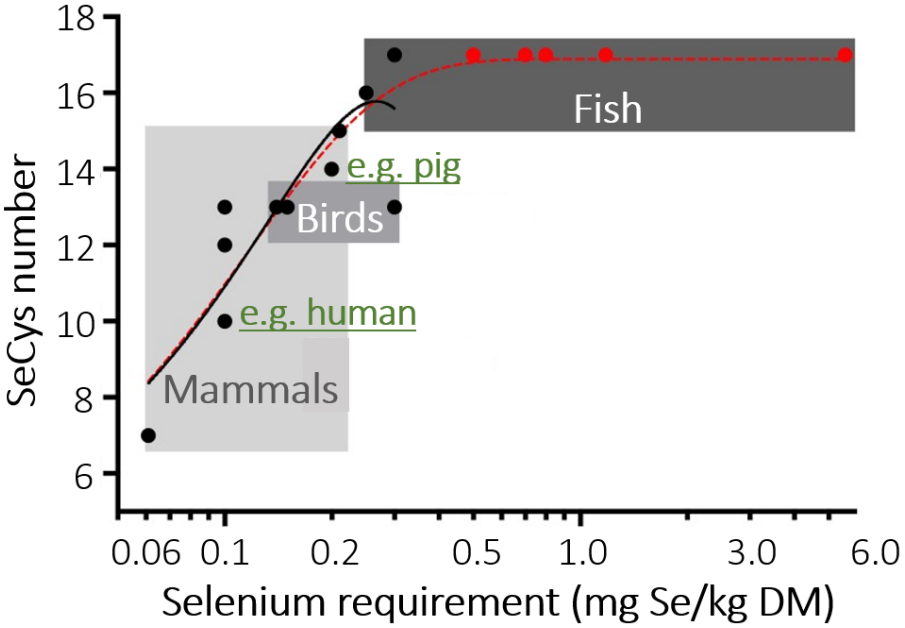

Figure 2 Selenium requirement of a species *in relation to the SeCys content of its selenoprotein P (modified from Penglase et al. [205])*

Humans

The recommended human daily intakes range from 6-85 µg/d depending on the country, age, gender, and physiological status [76, 206]. The revised reference values for Se intake for Central Europe, calculated based on the saturation of SelP and reference body weights, are 70 µg/day for men and 60 µg/day for women [207]. However, the estimated dietary Se concentration

based on the typical household consumption ranges between 30 and 110 μg/day in various European countries [140, 208, 209].

In some regions of the world, such as New Zealand's South Island, parts of China, and the majority of European countries, Se intake from local food may not be adequate to ensure optimal activity of protective selenoenzymes [76]. An adequate human Se intake in countries with Se-deficient soils is often ensured by imported grain. In several European countries, e.g., Norway, the Se intake has declined during the last decades owing to reduced import of Se-rich wheat from North America and Canada [70, 210-212].

Pigs

The pig has 14 Se atoms in its SelP amino acid chain [213], leading to a predicted Se requirement of 0.17±0.05 mg Se/kg feed dry matter (DM) according to Penglase et al. [205]. This is in line with recommendations for pigs around 20-25 kg and above [214, 215] (see Tables 4 and 5).

The current dietary Se requirements for pigs, as established by the National Research Council (NRC) on Swine Nutrition, are presented in Table 4 and range from 0.3 mg Se/kg in nursery pigs to 0.15 mg Se/kg in finishing pigs and breeding sows [214]. Within the European Union, the total level of dietary Se when supplemented with inorganic Se sources is limited to 0.5 mg Se/kg [216]. Dietary Se from organic Se sources is limited to 0.2 mg Se/kg feed. The Danish recommendations are similar to those made by NRC (see Tables 4 and 5) [214, 215].

Selenium supplementation in Norwegian swine feed

Schrauzer [217] stated that Se should be supplemented in forms in which it occurs in major staple foods. Foods and feed naturally contain organic forms of Se, with the inorganic forms only entering the diet as supplements or contaminants [84].

A schematic timeline over supplementation of feed is presented in Figure 3.

Sodium selenite (selenite)

Inorganic Se salts are not nutritional forms of Se [217] as Se normally enters the food chain through plants or meat [67] containing mainly Se in the form of SeMet.

In 1970, the inorganic Se salts selenite and sodium selenate, were approved as feed additives up to 0.5 mg Se/kg complete feed in Europe [27], but Norway first began to add 0.15 mg selenite/kg feed [218] in 1980. In 1983, Norwegian pelleted pig feed contained on average 0.34±0.09 mg Se/kg DM [218]. From 1984/5, pig feed was enriched with 0.20 mg Se/kg [165, 187].

Subsequently, the Se concentration in liver tissue from pigs increased from around 0.35 µg/g wet weight (WW) before 1980 to more than 0.50 µg/g WW on average around 1985 [218]. The incidence of NMD decreased notably in well-managed farms. According to Frøslie et al. [218], the incidence of MHD did not decrease in the same manner. Liver Se concentrations found in MHD pigs are usually between 0.20-0.40 µg/g WW and often not considered deficient [218]. It seemed that this disease appears at higher Se levels than other Se-and-vitE-responsive conditions and could have a multifactorial aetiology [201]. It has been stated that vitE plays a significant role in the development of MHD [183, 219].

Table 4 American recommendations (National Research Council, NRC) regarding Se and vitE supplementation in swine feed [214]

	Body weight range (kg)							**Sows**	
	5-7	7-11	11-25	25-50	50-75	75-100	100-135	G**	L**
Requirements per kilogram of diet									
Se (mg)	0.30	0.30	0.25	0.20	0.15	0.15	0.15	0.15	0.15
vitE (IU)*	16	16	11	11	11	11	11	44	44
Requirements (amount per day)									
Se (mg)	0.08	0.14	0.23	0.30	0.32	0.38	0.42	0.31	0.89
vitE (IU) *	4.3	7.5	10.0	16.5	23.3	27.6	30.7	92.4	262.5

*1 IU vitE=0.67 mg of D-α-tocopherol or 1 mg of DL-α-tocopherol acetate;
**G - gestation, L – lactation

Table 5 Danish recommendations for Se and vitE supplementation for swine [215]

	Body weight range (kg)				
	6-9	9-30	30-105	>105 incl. sows G**	Sows L**
	Requirements per feed unit of diet				
Se (mg)	0.35	0.35	0.2	0.2	0.2
vitE (IU)	140	140*	40	40	165
As DL-α-tocopherol (mg)	130	130*	36	36	150
As all-rac-acetate (mg)	140	140*	40	40	165
As RRR, mg	94	94*	27	27	111
As RRR^ acetate, mg	103	103*	29	29	121

*By applying mixtures of 20-30 kg, vitE can be reduced to the same level as for slaughter pigs. 130 mg DL-α-tocopherol/feed unit are documented for the period 6-20 kg. Recommendations for vitE depend on the form applied by feed, as the relative bioavailability is different [220, 221]; **G - gestation, L – lactation; ^2R,4'R,8'R-alpha-(5,7-(C2H3)2) tocopheryl acetate (d6-RRR-alpha-tocopheryl acetate): α-Tocopherol with natural stereochemistry of the three chiral carbons

Organic selenium sources - selenized yeast (Se yeast) and selenomethionine (SeMet)

Organic Se has a higher bioavailability than inorganic Se [76, 85, 222-224], which is due to the non-specific incorporation of SeMet instead of methionine into non-seleno body proteins in, for example, skeletal muscle tissue, erythrocytes, and plasma [76, 225] (see Figure 1).

As the first organic Se source, a Se yeast was approved by the EU as a Se supplement in 2006 at a dietary level of 0.5 mg Se/kg feed (see Figure 3) [226]. Admittedly, several researchers have been concerned because of poorly characterized organic Se supplements, which could potentially cause a build-up to toxic-level Se in tissues [76, 78, 204]. As a consequence, the addition of organic Se was reassessed by the European Food Safety Authority and, in 2011, restricted to 0.2 mg Se/kg complete feed to ensure consumer safety (see Figure 3) [204]. In the same year, the Norwegian feed industry started to add Se yeast to the sow feed.

Good-quality Se yeast preparations should not contain more than 1% inorganic Se, the remainder having been converted into organic Se species or removed from the final product [76, 227].
Variable Se concentrations and Se species in Se yeast may relate to the yeast strain, and the difference between the processes applied to prepare the Se yeast [76, 177, 232, 233]. However, SeMet concentration in Se yeast purchased from reputable manufacturers operating good quality control and quality assurance schemes should be stable [76]. The lower bioavailability compared with pure SeMet preparations presumably reflects the fact that the yeast has to be digested and that not all Se in Se yeast is SeMet [76]. Absorption and retention of Se from Se yeast in humans ranges respectively between 54–90% [234] and 60–75% [235] depending on the Se yeast used.

In 2013 the first pure SeMet source was authorized by the EU [236] and in 2014 in Norway [231]. Until spring 2016, organic Se (Se yeast) was used only in the concentrate for high producing animals like sows in Norway. Now, organic Se (Se yeast and pure SeMet sources) is also increasingly used in Norwegian swine feed for piglets and grower-finisher pigs.

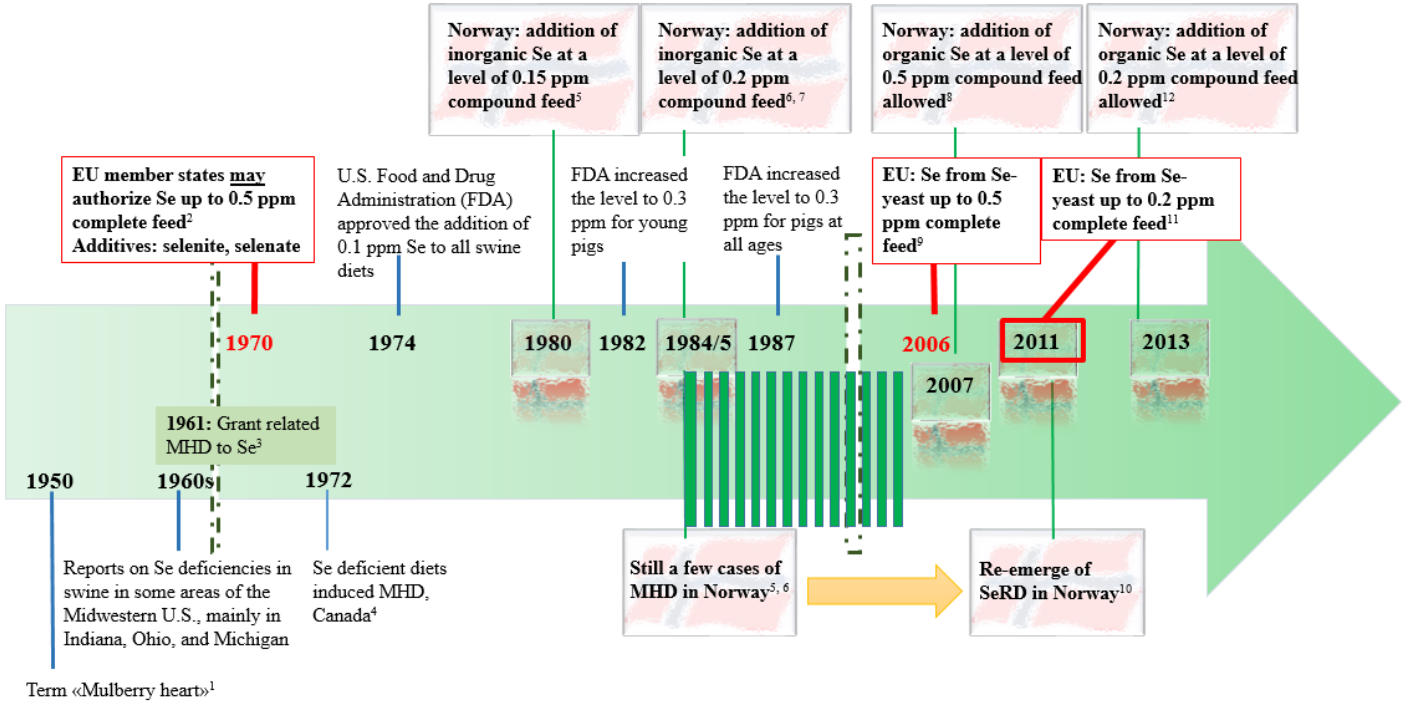

Figure 3 Rough timeline of selenium supplementation to pig feed in the EU, Norway, and the USA *in relation to the occurrence of selenium/vitamin E responsive diseases in pigs*

References: [1]Lamont et al. 1950 [228]; [2]The Council of the European Communities 1970 [27]; [3]Grant 1961 [184]; [4]Sharp et al. 1972 [229], [5]Frøslie et al. 1985 [218]; [6]Frøslie 1991 [165]; [7]Øvernes and Frøslie 1992 [187]; [8]Mattilsynet [Norwegian Food Safety Authority] 2007 [230]; [9]European Food Safety Authority (EFSA) 2006 [226]; [10]Norwegian Meat and Poultry Research Center 2012 [193]; [11]EFSA 2011 [93]; [12]Mattilsynet [Norwegian Food Safety Authority] 2013 [231]

Selenium storage in the organism

Feed supplemented with organic Se either as Se yeast or SeMet has proven to elevate tissue Se concentrations in both sows and their offspring more efficiently than inorganic Se [202, 237]. However, SeMet is transferred from the digestive tract into the body without being recognized as Se [238, 239].

After a certain period of SeMet (or Se yeast) intake, a steady state may be reached wherein turnover from the general protein pool can supply SeMet [217] at a level associated with beneficial health effects [140]. As an example, administration of 200 µg Se yeast/d to humans increased plasma Se up to about three months, after which the concentration levels off and reached a clear plateau at around 190 µg/L by one year [76]. This plasma Se level is well below that associated with toxicity (1054–1854 µg/L in whole blood, generally 23% higher than in plasma) [240].
Free SeMet can be transselenated to SeCys, which is then converted to H_2Se by a β-lyase [92], the starting point for the selenoprotein synthesis (see Figure 1). Thus, humans or animals supplemented with organic Se can maintain higher activities of selenoenzymes during Se depletion for longer periods than those supplemented with inorganic Se [76]. The ratio between the incorporation of SeMet into body proteins and the formation of alternative, more active metabolic products, depends on the amount of dietary methionine and the SeMet intake [76, 241]. Also, organic Se is more effectively transferred to breast-fed infants or suckling animals than inorganic Se, thereby preventing the risk of deficiency [76].

Contrarily to Se from organic sources, Se as selenite can be directly incorporated in selenoproteins [242], but cannot be stored in the body except in selenoproteins [222]. Reported whole body half-lives of SeMet and selenite in humans are 252 and 102 d, respectively, implying that SeMet is retained 2.5 times longer in the body than selenite [243]. The above-mentioned storage in tissues and physiological protein catabolism gives SeMet a slower

whole-body turnover rate [76] maintaining an increased Se status, thus acting as a potential reservoir for Se [244].

In Se-replete rats and humans, the Se distribution has been described as followed: kidneys > liver > testes > heart > skeletal muscle > brain [245]. In cases of Se deficiency, Se concentrates in the testes, brain, thymus, and spleen at the expense of other tissues such as, for example, muscle tissue [246].

One-health and human Se intake

Besides animal welfare, it is important to mention the one health aspect of Se. The Se concentration in food products and forages is highly variable [164, 247] depending on the plants used or, respectively, their soil of origin, climatic conditions, cultivation, breeding methods, and methods of preparing feed and food products [248, 249]. Measures like import of Se-rich wheat, applying Se-enriched fertilizers, and supplementing animal feed with Se have resulted in higher Se concentrations in meat and poultry and thereby increased the human Se intake particularly in, i.e., New Zealand and Finland [250-252].

Se in food products most often occurs in combination with proteins: thus, products with high protein content may have a higher Se concentration [248]. These products include mushrooms, meat, marine products including fish, offal, Brazil nuts, and cereals [247, 253, 254]. Se levels in dairy products are negatively correlated with fat content [248]. In line with a low content of protein and high content of water, fruits and vegetables contain only small amounts of Se [248]. In conclusion, the Se concentration in foods can be described as follows: mushrooms such as shiitake > organ meats and seafood > muscle meats > most agricultural crops and related products > dairy products > fruits and vegetables [162, 255, 256].

Since meat is an important source of Se in human nutrition, Se enrichment of pig feed may be one way of improving the Se status in the Norwegian population [218]. It has been shown that supplementation of farm animal

diets with organic Se instead of inorganic Se increases Se levels in meat and thus in non-vegetarian humans [257, 258].

Vitamin E

The term vitE comprises a group of methylated phenolic compounds known as tocopherols and tocotrienols. The biologically most active form of vitE is α-tocopherol. The absorption and transport of this antioxidant are closely linked with that of lipids [259].

VitE is a major chain-breaking antioxidant in blood plasma and cell membranes [260-262]. It oxidizes the oxygen singlet, takes up free radicals (e.g., OH•), neutralizes peroxides, and captures $^{\bullet}O_2^-$ to convert it into less reactive forms. VitE is considered the most important lipid molecule protector because it protects the polyunsaturated fatty acids (PUFA) of cell membrane phospholipids from cellular peroxidation, and it inhibits the peroxidation of low-density lipoproteins (LDL) [259, 260, 262, 263]. Erythrocytes are at higher risk of damage from peroxides than most other cells due to their high concentration of oxygen [264], and erythrocyte membrane fluidity is enhanced by the presence of PUFA. In sheep erythrocytes, vitE has been demonstrated to act as a membrane-stabilizing agent, independently of its antioxidant properties [264]. In an in-vitro study, pig erythrocytes exhibited the lowest resistance to oxidative stress compared with ducks and chickens [265].
Compliance with the recommended daily vitE intake appears to protect against cardiovascular disease, due to protection of LDL from oxidation, one of the main risk factors of this condition [266]. To avoid MHD and NMD, but also *Hepatosis dietetica*, in feed-efficient pigs with a high ADG and feed efficiency, the Norwegian feed industry adds high levels of vitE since legislation limits the Se supplementation to feed. Despite actual recommendations by the NRC (see Table 4), vitE supplements above 100 mg/kg as DL-α-tocopherol-acetate are common in feed for weanling pigs in Norway, which is in accordance with the recommendations of Moreira and

Mahan [267] for diets without the addition of fat [30]. The usage of vitE/kg feed in piglets ranged from 30 mg for Brazil and China to 140 mg for Great Britain, with a global, non-weighted mean of 69 mg [220]. For Western countries, the mean was close to 75 mg vitE/kg feed [220]. VitE is one of the least toxic lipid-soluble vitamins. However, high doses of vitE have been shown to drastically induce catabolism of vitE to avoid excessive accumulation, resulting in a shorter half-life [220].

Jensen et al. [268] showed that the porcine liver has a very large capacity for short-term storage of vitE and that peak values are reached in the liver (Table 6). The response to dietary vitE changes was fast in porcine serum and liver but slower in skeletal muscle and adipose tissue [268, 269]. Thus, the vitE concentration in skeletal muscle and adipose tissue, as opposed to the liver, will reflect the long-term nutritional history [268].

Table 6 Overview of results obtained by Jensen et al. [268] in a study conducted on grower-finisher pigs[1].

	Depletion I*	**Supplementation****	**Depletion II after 2d*****	**Depletion II after 7d**
Serum (mg vitE/l)	0.3-1.1	4.8-10.2		2.6±0.4
Liver (mg vitE/kg^)	0.8±0.2	102.9±26.2	17.8±3.1	6.0±0.9
Adipose tissue (mg vitE/kg^)	2.8±1.0	31.6±10.8	No decrease	No decrease
Skeletal muscle^^ (mg vitE/kg^)	0.5±0.2	6.8±1.0	No decrease	No decrease
Myocardium (mg vitE/kg^)				13.6±1.5

[1]as α-tocopherol; *Four weeks on a basal diet containing 16 mg vitE/kg; **Seven weeks on a same diet with 405 mg vitE/kg; ***One week on a basal diet containing 16 mg vitE/kg; ^Wet tissue; ^^*M. biceps femoris*

The liver preferentially incorporates vitE into lipoproteins that are released into the bloodstream for distribution to peripheral tissues [270], which might at least partly explain the data obtained by Jensen et al. [268] (Table 6).

Knowledge gaps at the time of project initiation

The project "Selenium requirement in Norwegian pig production" (NRC project no. 233658, Matfondavtalen) was initiated on the background of a Research Summary accepted by the Research Council of Norway, and later of results from the Norwegian Meat and Poultry Research Centre's (Animalia) survey of their contractual veterinarians in Norwegian breeding units [193]. Although this issue had been widely investigated, and dietary Se and vitE levels were thought to be optimized, Se-and-vitE-responsive diseases seemed to re-remerge in feed-efficient, and fast-growing pigs in Norway at all production levels. In Norway, pelleted pig feed has mainly been enriched with inorganic Se as selenite. Thus, one hypothesis was that Se requirements in these modern pigs with large muscle mass have increased. The second one was that the use of organic Se might mitigate the occurrence of Se-and-vitE-responsive diseases, increase the sow's Se reservoir and the Se transfer to the offspring, and subsequently improve the weaned piglets' Se status more efficiently than selenite.

Aims of the study

The primary objective of this knowledge-building project was to compare the effects of L-selenomethionine (SeMet; organic Se) and selenite as sources of Se in Norwegian swine feed on pig health, and Se retention in porcine tissues and bodily fluids. To achieve this objective, the project focused on the following secondary aims:

- Se requirements: Evaluate the influence of different Se sources in pig feed with special reference to the maximum allowed levels of Se supplementation in the EU legislation and dietary Se levels slightly above the maximum limit, on production parameters in pigs.
- Se retention and transfer: Study different dietary Se sources and levels and identify the best suited to optimize Se retention in porcine tissues as well as Se transfer from sows to piglets (determination of total Se and its Se species in heparinized plasma, colostrum, and milk).
- Animal health: Examine clinical health, haematology and clinical biochemistry in grower-finisher pigs, sows, and their offspring, as well as gene expression in whole blood and skeletal muscle in grower-finisher pigs, including studies of acute oxidative stress caused by systemic LPS-challenge, after feeding different Se sources and concentrations.

Summary of papers

Paper I

A descriptive report of the selenium distribution in tissues from pigs with mulberry heart disease (MHD)

From 2015 to 2017, documented MHD field cases were collected and tissue Se concentrations were compared with those from controls. In this study, eight MHD cases from commercial farms and a pet pig producer located in the southwest and eastern regions of Norway, as well as three control animals originating from these farms were included. Organ and tissue Se concentrations were determined to characterize the Se distribution and to identify any differences between MHD cases and controls.

MHD cases and controls were weaned pigs with average BW of 17 kg (range 9 to 46 kg BW) and one pet piglet (Mangalica, 6 kg BW). Se concentrations were determined in samples from the cardiovascular, respiratory, digestive, urinary, immune, endocrine, muscular, and integumentary systems using inductively coupled plasma-mass spectrometry (ICP-MSMS). All pigs with MHD suffered sudden deaths. Control animals were euthanized without being bled before necropsy and sampling. Significant different mean Se concentrations between MHD cases and controls were found in cardiac samples and nine of ten skeletal muscles analysed. Based on the samples from all ten different skeletal muscles, mean Se concentrations in MHD cases were 0.34 (0.01) mg/kg DM compared with 0.65 (0.02) mg/kg DM in control pigs. In cardiac samples, mean Se concentration from MHD cases was 0.87 (0.02) mg/kg DM vs. 1.12 (0.04) mg/kg DM.

Additionally, significantly lower Se concentrations compared with controls were found in samples from the thymus, gastric ventricle, duodenum, jejunum, caecum, liver, kidney, and skin.

Based on the present work, the currently common practice regarding tissue analyses in MHD cases could be refined including samples from the muscular

system. The evident differences in mean Se concentrations could make such samples relevant for complementary measurements of Se concentrations to help confirm the MHD diagnosis. Despite the limited number of sampled pigs being different in terms of genetics, size, and feeding regimes, the variation of Se concentrations in each organ and tissue was low between MHD cases. Since this report includes a limited number of MHD cases and controls, the results should be corroborated by a controlled, larger study.

Paper II

Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs

The study was conducted in two parallel trials including 24 grower-finisher pigs to compare effects of the dietary Se sources selenite, Se yeast or SeMet and one Se-deficient control diet on the expression of selected genes in whole blood and tissue from one skeletal muscle, haematological and clinical biochemical parameters, and muscle morphology. Se concentrations were measured in heparinized plasma and several tissues. From 12 pigs, muscle samples obtained from *M. long. dorsi* were examined before, 6 weeks into the trial and at the end (after 9 weeks) of the trial. The remaining 12 pigs were challenged once with LPS intravenously 6 weeks into the trial and euthanized 24 h after injection.

Transcriptional analyses of *M. long. dorsi* showed that the selenogenes *SelenoW* and *H* were higher expressed in pigs fed Se-supplemented diets compared with control. Furthermore, the expression of *interferon-gamma* and *cyclooxygenase 2* was lower in the Se-supplemented pigs versus control. In whole blood samples before LPS exposure, *SelenoN*, *SelenoS,* and *thioredoxin reductase 1* (*txnrd1*) were expressed to a greater extent in pigs fed selenite-supplemented feed compared with the other groups. After LPS exposure, mRNA levels of *gpx1* and *SelenoN* were more reduced in pigs fed selenite compared with pigs fed organic Se. No significant effects of Se source were found on haematological parameters or microscopic anatomy. The Se concentrations in various skeletal muscles and cardiac muscle were significantly different between the groups, with the highest concentrations in pigs fed SeMet, followed by those fed Se yeast, selenite, and control diet.

Products of most abovementioned genes are intertwined with the antioxidant system. Consistent with previous reports, these results indicate that dietary Se at adequate levels can support the body's antioxidant system. However,

data on gene expression in blood from selenite-fed pigs isolated before LPS exposure possibly indicate a higher level of oxidative stress due to selenite metabolism. Based on the results, it was suggested that muscle fibres of pigs fed organic Se are less vulnerable to oxidative stress compared with the other groups.

Paper III

Effects of dietary sodium selenite and L-selenomethionine enrichment on selenium concentrations in blood, colostrum and milk, feed intake, and effect on haematological and biochemical parameters in high-yielding sows

A field study in 32 periparturient sows fed different dietary concentrations of either selenite or SeMet was conducted to describe some key Se species, namely SelP, selenoalbumin (SeAlb), and SeMet as well as total Se in plasma, colostrum, and milk. In addition, feed intake, haematological and biochemical parameters were measured.

Thirty-two sows were allotted to four treatments from 30 d prepartum throughout, on average, a 32-d lactation period. Selenite-supplemented diets contained 0.40 and 0.60 mg Se/kg feed, while SeMet-supplemented feed contained 0.26 and 0.43 mg Se/kg feed. Concentrations of selenite and SeMet in complete feed exceeded the upper limits for total dietary Se and added organic Se in the higher supplemented diets, respectively, according to the EU legislation. Blood samples were collected at initiation of the study, at farrowing, and at weaning. Colostrum samples were collected at farrowing and milk samples at weaning. Total Se was determined using mass spectrometry (ICP-MSMS). Se species were separated and quantified using liquid chromatography in tandem with ICP-MSMS.

The SeMet-supplemented diets resulted in higher feed intake and higher levels of total Se, SelP, SeAlb, and SeMet in colostrum compared with selenite-fed sows. Similar results were obtained for levels of total Se and SeMet in milk at weaning. The higher dietary selenite concentration in sows' feed did not increase the Se transfer into colostrum or milk when compared with those receiving the lower level of selenite. However, an increase of serum zinc (Zn) from initiation until farrowing, observed in SeMet-fed sows, as well as a higher glutamate dehydrogenase (GLDH) activity in selenite-supplemented

sows in this period, might indicate a higher requirement of antioxidant defence in these sows.

To the researchers' knowledge, the present data on Se species in plasma, colostrum, and milk of sows represent the most complete investigation of Se in sows conducted to date. A higher amount of the above-mentioned Se species in the colostrum of sows supplemented with SeMet might strengthen the piglets' antioxidant system and passive immunity as well as improve their ADG. The higher feed intake in sows fed diets supplemented with SeMet is an interesting finding that warrants further investigation.

Paper IV

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

In this field study, piglets from the above-described sows were monitored from birth to 38 d of age. The trial diets herein were supplemented with Se similarly to the sows' diets. Effects on weight gain, various haematological and biochemical variables as well as heparinized plasma concentrations of vitE, total Se and Se species in the offspring were evaluated throughout the suckling period. Selenite-supplemented feed at 0.4 mg Se/kg (NaSe-0.4) represents a common Se source and -level in pig feed and therefore constituted the control diet of this study.

From 5 d of age, piglets from sows fed NaSe-0.6 had significantly higher mean BW than offspring from sows fed NaSe-0.4. At age 24 d and further on, also piglets in SeMet-groups had higher BW compared with piglets from sows fed NaSe-0.4.

Haematological and clinical biochemical results of concern were as follows: neonatal piglets in group SeMet-0.4 had significantly lower red blood cell (RBC) counts, haemoglobin (Hgb), and haematocrit (Hct) concentrations compared with piglets from sows fed NaSe-0.4. Additionally, a higher γ-glutamyl transferase (GGT) activity was observed in neonatal and 5 d-old piglets in group SeMet-0.2 compared with coeval piglets in group NaSe-0.4. Furthermore, at age 5, 24, and 38 d, group NaSe-0.6 excelled with increased specific haematological variables culminating at age 38 d with increased Hct, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) as well as increased activities of aspartate transaminase (AST) and lactate dehydrogenase compared with other groups.

Generally, offspring from SeMet-fed sows had higher total Se plasma concentrations than those from selenite-fed sows. At each point in time, there was a dose-related effect on plasma SeMet concentrations in piglets from

SeMet-fed sows. Besides, these piglets had higher plasma levels of GPx3, SelP, and SeAlb at several points in time.

Maternal supplementation with selenite during gestation influenced haematology and clinical biochemistry in piglets somewhat differently than SeMet-enriched diets. Growth performance was influenced by both Se source and dietary Se level. The higher weight gain in piglets from NaSe-0.6-fed sows might relate to the higher Hct as erythrocytes are essential to provide the rapidly growing cells with sufficient amounts of oxygen. Higher haematological parameters might be a sign of increased selenoprotein synthesis improving erythropoiesis. However, results on some enzyme activities might indicate that piglets from sows fed NaSe-0.6 had to cope with increased levels of oxidative stress compared with those originating from SeMet-fed sows or lower dietary levels of selenite. Offspring from sows fed SeMet-supplemented diets was probably better protected against birth- or growth-related oxidative stress due to higher plasma GPx3 levels and thereby better prepared for situations of increased Se requirements as indicated by higher weight gain.

Methodological considerations

The thesis is based on three animal trials (see Table 7), all of which were dose-response trials. In all trials, clinical and laboratory-based methods (see Table 9) were applied and are described in detail in **Papers I-IV**. In the following section, all parts of the study have been fully evaluated to the best of my knowledge.

Experimental design

The descriptive study of MHD (**Paper I**) was based on pigs from several commercial pig farms. To ensure good sample quality, pigs were necropsied the day they were found dead, and samples were isolated, weighed, packed and frozen by the same person. Information on the pig's life including feed, age and hybrid were gathered from the corresponding farmer. Drawbacks herein were that all pigs included were raised under different management regimes and not under experimental conditions. This made it impossible to control variables like feed composition, feeding regime, hygiene, ventilation, room temperature, size and quality of pens. However, the study offered a unique possibility to outline the situation in Norwegian pig production regarding Se deficiency-related problems.

Two feeding trials (**Papers II-IV**), each including four diets, were conducted. In the grower-finisher trial (**Paper II**), pigs were allocated to four groups in an independent measure design. The control group received a diet not enriched with Se, whereas the three other groups received diets enriched with Se at the dietary level of about 0.3 mg Se/kg feed each from a different Se source (selenite, Se yeast, SeMet).

All parts of the sows-offspring trial (**Papers III and IV**) were designed as two-by-two factorial experiments. Here two Se sources, the inorganic selenite and organic SeMet, were each added at two different dietary levels. The intention here was to compare legal and commercially applied dietary Se concentrations (here 0.40 mg Se/kg feed enriched with selenite and 0.26 mg

Se/kg from SeMet) with enrichments above the legal limits (0.60 mg Se/kg from selenite and 0.43 mg Se/kg from SeMet). EU-legislation allows different limits depending on the chemical nature of the Se supplement: 0.5 mg Se/kg feed from inorganic supplements like selenite [216] and 0.2 mg Se/kg from organic Se sources like SeMet [204]. It would have been advantageous to compare similar dietary Se concentrations of both sources applied, but then low levels of selenite had to be added or quite high levels of organic Se.

In addition, the grower-finisher pigs (**Paper II**) were challenged with a single intravenous LPS-treatment six weeks into the grower-finisher phase. This LPS-study was conducted in time-series design in which each individual served as its own pre-treatment control. Unfortunately, the experimental groups were small (three pigs per feeding group) making the results highly variable. However, using same age, gender and hybrid of pigs decreased the risk of eventual within-individual variation.

Animals

Ethical considerations

The Norwegian Food Safety Authority approved all trials including the LPS study, and performances complied with the current European 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).

A high standard of ethical consideration was applied following the Three Rs (3Rs; *Reduction, Refinement, and Replacement*), defined by Russell and Burch [271] as guiding principles for the more ethical use of animals in testing. Replacement of live pigs was impossible as this study aimed to evaluate the impact of Se in animal feed *in vivo*.

Some procedures, e.g., snaring or blood sampling, were shown to produce a higher level of stress, whereas others such as ear tagging induced more moderate stress [272]. Following the 3Rs [271], we sought to reduce the

number of animals per feeding group exposed to stress and discomfort, e.g. restraining and blood sampling (Table 7). However, ear tagging was indispensable as it enabled monitoring of each animal individually throughout the trials.

Table 7 Overview of numbers of pigs per study including number of pigs restrained and pigs excluded

Trial*	Paper	No. of pigs included	No. of pigs restrained repeatedly	No. of pigs excluded due to health issues
GFT	II	24	24	0
SOT-Sows	III	32	31	2
SOT-I	IV	483/128^	72	0
SOT-II	V	128	72	2

*Grower-finisher trial (GFT), sows-offspring trial – sows (SOT-sows), sows-offspring trial – offspring (SOT-I); ^A larger number of piglets were weighed right after birth

Only subgroups of the pigs included in the separate trials were sampled. The size of subgroups sampled was thereby large enough to overcome the effects of normal biological variance and thus to avoid the necessity of eventual repetitions due to unreliable results. Refinements included the application of pain and distress relieving drugs during biopsy-procedures and the definition and use of humane endpoints throughout the study. Other stressful moments, like weaning and transport to the research facility [273-275] were not preventable, but weaned pigs were mixed litter-wise to alleviate weaning stress.

Confounding factors

The animals sampled in the MHD study (**Paper I**) came from different commercial facilities in two different counties of Norway causing differences in management, e.g., feeding routines including different feed mills. All pig producers included were experienced and used standard pig feed. Age and thus body weights of the included animals varied greatly. One pig received

sow milk only. Both age (e.g., liver, kidney) and feed (inorganic vs. organic Se) can influence Se concentrations in a given tissue.

In all feeding trials (**Papers II-IV**), animals were acclimatized to the research facilities for one week after arrival. Trained staff controlled the animals' general condition visually several times a day. Housing conditions potentially influencing the animals' well-being, or the experimental outcomes were optimized. To minimize disturbance by environmental factors, the pigs in a trial were housed together, received the same basal diet, and were handled by the same persons. As far as possible, the animals originating from one sow were allocated to the same group. Unfortunately, pigs included in the grower-finisher trial (**Paper II**) came from two different commercial facilities (facility 1 – LPS-study; facility 2 – Isolation of muscle samples). Gender as a confounding factor was eliminated in the grower-finisher trial (**Paper II**) and, of course, in the study on sows (**Paper III**). Males and females from the sows' offspring were equally distributed over all diet groups (**Paper IV**). Cross fostering was conducted to equalize litter size, as far as possible within the same feeding group. Piglets moved to sows of another feeding group were excluded from the trial.

Feeding groups

In the grower-finisher trial (**Paper II**), one group of pigs received a Se-deficient diet (control group). This was impossible in the sows-and-offspring trial (**Papers III and IV**) since the first two parts of this trial were conducted as a field trial in a commercial facility. Feeding Se-deficient diets to sows would pose a significant risk of decreased litter size and other reproduction-related problems as described elsewhere in this document.

Unfortunately, neither total Se nor vitE were measured in the feed used at the commercial farms included in the MHD study (**Paper I**). Since all farms received a full feed from certified feed mills, it was assumed that the feed was well formulated according to the above-mentioned EU guidelines. Also, as described for MHD, only individual pigs in good to exceptionally good

condition died suddenly, whereas the majority of the pigs in the same group were unaffected.

For **papers II-IV**, the regressions (R *lm*) were repeated so that all diets were used as control.

Sampling

Major influencing factors on the quality of the collected specimens were handling of both the animal during sampling and the collected sample until analysis. Isolation, preparation, and analysis increased the risk of sample-to-sample variation due to the human factor. This factor was minimized due to the handling of all samples in a short time and by the same persons per method, minimizing human-to-human variations.

Whole blood sampling

All blood samples were drawn from the jugular vein using the Vacuette® system (Greiner Bio-One, Austria). Depending on the age of the pig 2, 3, or 6 mL Vacuette® Lithium Heparin tubes were used.

After haematological analysis, heparinized blood samples were centrifuged, and gained plasma was kept frozen at –20 °C in plastic vials. On the opposite to serum, plasma contains coagulation factors. The limiting factor in the trials presented here was the sampling of neonatal piglets and the subsequently limited withdrawable blood volume. At the time of planning, 2 mL Serum clot activator tubes were not available. Since none of the parameters of interest were affected by coagulating factors, plasma was chosen as sample material.

During the LPS-studies, blood samples for subsequent RNA-isolation were drawn using PAXgene®-tubes (2.5 mL) and frozen at –20 °C. After 24 h, they were transferred to –80 °C until analysis.

Handling and LPS stress led to severe haemolysis in some plasma samples. Those were excluded from the biochemical analysis.

Time points for blood sampling

Before initiating the feeding trials and before the LPS challenge, whole blood samples were drawn from pigs in all studies. These samples served as baseline samples for haematology and the gene expression study. The heparinized plasma was used for both clinical biochemistry and measurements of total Se as well as Se species.

In the sows-and-offspring trial, blood and colostrum samples were isolated from the sows at farrowing, four weeks into the feeding trial, enabling a comparison of Se status and Se species in maternal blood and colostrum (**Paper III**) with the status in neonates (**Paper IV**). Subsequent blood samples from piglets were isolated after colostrum intake (5 d of age), at 24 d of age to reflect the piglets' status at an internationally commonly practiced weaning age and on day 38 to reflect values from piglets experiencing post-weaning stress (**Paper IV**). Piglets in this study were weaned at 31.6 (1.3) d which is close to the Norwegian average of 33.3 ± 0.3 d (2011-2020) [59, 60].

After six weeks into the grower-finisher trial (i.e., at 16 weeks of age and immediately before the LPS challenge), samples were drawn reflecting the time of a stable Se status under the different dietary conditions (**Paper II**). In the LPS trial, blood samples were isolated before LPS administration and 1*, 3, 5 and 24* h after LPS administration (*with PAXgene®-tubes).

Tissue samples

MHD study

Thirty-two tissue samples collected from pigs were included in this study (**Paper I**). Tissues were sampled from possible MHD cases after the macroscopic diagnosis of MHD and from controls from the same farms after exclusion of MHD. The animals were included in the study after confirming the diagnosis by histopathological examination of cardiac tissue sections.

Feeding trials

Pigs were kept until they attained a live weight of around 107.9 (8.9) kg in the grower-finisher trial (**Paper II**). During this period in the grower-finisher

trial, samples from *M. long. dorsi* were isolated at three points in time (**Paper II**), before starting feeding the trial diets (biopsy), six weeks later (biopsy), and after slaughtering. From the biopsies, one sample per animal (around 25 mg) was fixed in RNAlater for subsequent RNA isolation. Three additional samples, together weighing around 75 mg, were stored frozen at -20 °C until Se determination.

Several tissue samples for measurement of Se and/or vitE concentrations were taken from stillborn piglets or piglets dying before colostrum intake (sows-and-offspring trial; **Paper IV**) and from pigs after slaughtering (grower-finisher trial; **Paper II**).

Methods

Feeding experiments

The feed applied in the grower-finisher trial (**Papers II**) was produced by the NMBU-owned Centre for Feed Technology (Fôrtek), but all the other feed was produced by a commercial feed mill. Since the batches produced for each group in these trials were small, it was a challenge to adjust the desired Se concentrations for each trial and group. In the sows-and-offspring trial (**Papers III and IV**), the Se levels were significantly different between diets, but because of the partly large variance in the batches, some of the diets had to be re-produced.

The Se supplement SeMet for all trials was supplied via the preparation Excential Selenium 4000, Orffa, Netherlands (**Papers II-IV**), and the Se yeast used in the grower-finisher trial (**Paper II**) was supplied by Alltech. The manufacturer of the preparation Excential Selenium 4000 guaranteed a Se concentration of at least 0.16% Se and at least 0.40% SeMet. The Se concentration and speciation of the Se yeast applied in the grower-finisher trial (**Paper II**) remained unknown.

Until entering the trial, pigs in the grower-finisher trial (**Paper II**) received selenite-enriched feed, not influencing the results in the Se yeast and SeMet-

supplemented groups. Unfortunately, the sows in the sows-and-offspring trial (**Paper III**), prior to entering the trial, were fed a diet enriched with selenite and Se yeast at a final dietary level of 0.4 mg Se/kg. This might have influenced results obtained from sows' plasma, colostrum, and milk (**Paper III**) and their piglets (**Paper IV**) as SeMet is continuously released from maternal body proteins during physiological protein turnover [10].

Lipopolysaccharide (LPS) challenge

Three grower-finisher pigs per diet were challenged with the same amount of LPS/kg BW after about six weeks on the grower-finisher diets (**Paper II**). LPS from *Escherichia coli* O111:B4 (L4391, Sigma-Aldrich, USA) in form of lyophilized powder was dissolved in a physiologic saline solution and given intravenously via *V. auricularis* at a concentration of 2 µg/kg BW. The pigs were euthanized and necropsied right after the LPS-study.

LPS, the major molecular component in the outer cellular membrane of Gram-negative bacteria [276] has been widely used in various production animal species to imitate bacterial infections [277]. In pigs, this model has been applied to study both the immune response [278-282], sickness behaviour [283, 284], and the mode of action of nutritional supplements [278, 285]. A study conducted by de Groot et al. [286] using female piglets aged 8 weeks showed that a dose of 2 µg LPS/kg BW induced consistent fever responses and sickness behaviour. Application of LPS induces oxidative and immunological stress [287] leading to, e.g., changes in numbers and proportions of blood leucocytes [288] as well as the production of circulating inflammatory factors [289], which were measured throughout the LPS study using haematologic and molecular methods (**Paper II**).

Haematology and clinical biochemistry

Blood samples collected in Lithium Heparin tubes were subjected to multiparametric haematological analysis (ADVIA 120 Haematology System, Siemens Healthcare GmbH). Clinical biochemistry analysis using heparinized

plasma was conducted by applying the ABX Pentra400 benchtop analyser (Horiba). This set-up enabled analyses with little sample-to-sample variation.

Some plasma samples from piglets were high in fat causing interferences during analysis in the Pentra 400. In this case samples were diluted using physiologic saline solution.

Determination of selenium

Feed, heparinized plasma, colostrum, milk and tissue samples to be analysed for their Se concentration and speciation (**Papers I- IV**) were stored at -20 °C until preparation for analysis.

Total selenium

The total Se concentration was determined in feed, plasma, colostrum, milk, and tissue samples (**Papers I- IV**) by ICP-MSMS (Agilent 8800 and 8900). The ICP-MSMS method used herein is described in detail by Brandt-Kjelsen et al. [290] and in **Papers I-IV**. An ICP mass spectrometer is a highly sensitive detector with detection limits in the pg-ng range [291], well below the concentration in serum that is considered Se-deficient [292].

Before ICP-MSMS analysis, solid samples (i.e., feed and freeze-dried tissues) were microwave-digested with sub-distilled, ultrapure nitric acid at 260 °C for 25 min using an UltraClave or UltraWave system (Milestone); whereas plasma, colostrum, and milk samples were diluted with a mixture of butanol, EDTA, NH_3 and Triton X-100 (1:10 V/V). Tissue samples were freeze-dried (> 20 h) before digestion since dry mass is a more reliable measure of mass than wet mass.

Tellurium (Te) was used as an internal standard in samples containing organic residues, i.e., colostrum, and milk, whereas enriched ^{74}Se was used with plasma and tissue samples. Different certified reference materials (Table 8; **Papers I-IV**) were prepared and analysed in the same manner as the samples to be used as quality control materials to evaluate the accuracy and precision of the methods. Method blanks (n = 5) were used in each run to

calculate the limit of quantification (LOQ; as 10 times the standard deviation of the measured concentration in the method blanks).

Table 8 Standards and reference materials applied during determination of selenium

	Description	Manufacturer
Total Selenium		
Tellurium	Internal standard	
Se ICP reference solution	Se standard solutions	Inorganic Ventures, USA
^{74}Se (>99.9% enriched solution)	Internal standard	
1567a wheat flour	NIST standard reference material	NIST, USA
1570a trace elements in spinach	NIST standard reference material	NIST, USA
Trace elements serum L-1	Seronorm™ certified	SERO, Norway
Trace elements serum L-2	Seronorm™ certified	SERO, Norway
ERM® BD150 skimmed milk powder	European reference material	JRC, Belgium
ERM® BD151 skimmed milk powder	European reference material	JRC, Belgium
Selenium speciation		
Seleno-L-methionine (≥98% (TLC))	SeMet standard solutions	Sigma Aldrich, USA

Selenium speciation

In general, total element concentrations provide little or no information about mobility, biological uptake, and the eventual impact of elements within organisms [293]. The biological function of minor and trace elements is often determined by their association with specific proteins, peptides, and other species [294].

For measurement of Se species (**Papers III and IV**), speciation analyses were performed to separate and identify selenomolecules based on different physico-chemical properties.

The concentrations of different Se species in plasma, colostrum, and milk were determined by high-performance liquid chromatography (HPLC; Agilent

HP1260 liquid chromatograph, Agilent Technologies Inc.) in tandem with ICP-MSMS (O_2 mode; Agilent 8800) in time-resolved analysis mode (operating parameters and method details in **Papers III and IV**).

In a size fractionation step prior to chromatography, low (selenized amino acids) and high molecular-mass Se-containing species were separated by ultrafiltration using a 3 kDa cut-off membrane (Millipore Amicon centrifugal filters) and recovered in the filtrate and retentate, respectively. Free selenized amino acids (SeMet and SeCys) were determined using a reverse-phase liquid chromatography (RPLC) method that included a non-polar, silica-based C18 column (Agilent Poroshell 120 or Waters Atltantis T3) and a methanol mobile phase. High molecular-mass Se species (GPx3, SelP, and SeAlb) were separated using group specific affinity chromatography columns (HiTrap Heparin and HiTrap Blue columns; GE Healthcare) with ammonium acetate as mobile phase (**Papers III and IV**). GPx3 was not retained in either column, while SelP and SeAlb were eluted from the respective columns by changing the mobile phase. Both optimized chromatography methods allowed high-resolution separation of the Se species and combined with the sensitivity of the ICP-MSMS detection, resulted in very satisfactory quantification limits (i.e., LOQ of ca. 0.02 μg Se/L for selenized amino acids and ca. 0.04 μg Se/L for selenoproteins).

As for total Se analyses, replicate (samples and measurements) and certified reference materials were analysed for assessing precision, accuracy, and recovery of the entire analytical methodology from sampling to detection (full details in **Papers III and IV**).

Table 9 Overview of methods applied during all studies within the project

	MHD study	GFT*	SOT-Sows^	SOT-Offspring^^
Blood				
Haematology		X	X	X
RT-qPCR		X		X
Heparinized plasma				
Clinical biochemistry		X	X	X
Vitamin E			X	X
Total Se		X	X	X
Se speciation			X	X
Colostrum, milk				
Total Se			X	
Se speciation			X	
M. longissimus dorsi				
Muscle biopsy		X		
RT-qPCR – muscle		X		
Total Se		X		
Tissue samples, post-mortem				
Histology	X	X		X
Total Se	X	X		X
Feed				
Total Se		X	X	X
Vitamin E		X	X	X
Clinical parameters (LPS)				
Observation		X	X	X
Body temperature		X	X**	X
Heart-/Respiratory rate		X	X**	X

*Grower-finisher trial (GFT), ^Sows-offspring trial – sows (SOT-sows), ^^Sows-offspring trial – offspring (SOT-offspring); **Only conducted in case of reduced general condition.

Gene expression

RNA isolation

Total RNA from whole blood was isolated using the PAXgene®-products and the appendant protocol (PreAnalytiX®, UK) including some modifications (described in depth in **Paper II**). Total RNA from muscle samples (around 25 mg; **Paper II**) was extracted using RNeasy Fibrous Tissue Mini-Kit (Qiagen,

USA) following manufacturer's protocol and a Fastprep-24 (MP Biomedicals, Santa Ana, CA, USA) for tissue homogenization.

RNA is relatively unstable and rapidly degraded if not handled correctly. Handling of all samples was performed within a short time and by the same persons per method to minimize human-to-human variations. For all blood and muscle samples, RNA integrity was verified using a Bioanalyzer 2100 Expert (Agilent technologies, USA). Samples with an RNA integrity number (RIN) >7.5 were accepted for gene expression analysis. RNA purity and concentrations were measured using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). RNA was stored at −80°C until use.

LPS challenge leads to migration of leucocytes into the tissues causing a drop in circulating leucocytes in the bloodstream. This might have influenced the results in terms of the gene expression of some genes (**Paper II**), but the amount of RNA per sample was equilibrated prior to cDNA analysis giving similar amounts of cDNA.

Tissue samples were isolated at standardized areas to enable uniformity of the tissue samples.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) offers sensitive and rapid detection of transcriptional changes at the cellular level after the different treatments. RT-PCR is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement using reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR. RT-qPCR is used to quantitatively measure the amplification of DNA using fluorescent dyes. Unfortunately, results obtained on the basis of RNA cannot be used to unequivocal draw conclusions for the final level of proteins as regulation of transcript levels by post-translational control, e.g., mRNA turnover, mRNA surveillance, and regulation of translation are integral parts of gene expression [295]. Gry et al. [296]

showed that the correlation coefficients between levels of RNA and protein products of specific genes varied widely.

The RT-qPCR assays were performed according to MIQE standards [297]. First-strand cDNA was synthesized from 0.8 µg total RNA from all samples using SuperScript® III First-Strand Synthesis SuperMix for RT-qPCR (Invitrogen™) following manufacturer's protocol. Negative controls were performed in parallel by omitting RNA or enzyme. Protocols for RNA extraction and cDNA synthesis included a DNAse step to remove genomic DNA. Obtained cDNA was diluted 1:10 in molecular grade H_2O and stored at –20 °C. The RT-qPCR primers were obtained from the literature or designed using Primer3 (http://bioinfo.ut.ee/primer3/). The annealing temperature was optimized for each primer set. Their product was tested for amplification length using SDS-PAGE. The computer software belonging to the LightCycler 480 qPCR analysed fluorescent signals and determined the threshold value (quantification cycle, Cq) when the detection signal rose above the background level [298]. SYBRGreen was used as a fluorophore. Melting curves and non-template controls were evaluated after each run to monitor eventual contaminations and unspecific amplification or primer dimers. Samples were run in duplicates to account for pipetting errors. Mean normalized levels (MNE) of target genes were calculated from raw Cq values [299]. For target gene normalization, i.e. the calculation of the relative expression of the gene of interest (GOI), several stable-expressed reference genes (*rpl4* - 60S ribosomal protein L4, *hprt1* - Hypoxanthine(-guanine) phosphoribosyl transferase 1, *b2m* – β2-Microglobulin, *actb* – β-actin, and *gapdh* – Glyceraldehyde-3-phosphate dehydrogenase) were used [300].

Vitamin E-analysis

Vitamin E (as alpha-tocopherol) concentration in feed (n = 2 per diet), and plasma (N = 7 per diet) sampled at day 0, day 5, day 24 and day 38 of life was determined by RP-HPLC as described by Fuhrmann et al. [301] (**Paper IV**).

The main problem here was a possible contact of the samples with oxygen or other oxidizing factors during storage over several weeks prior to analysis, which might have decreased the vitE concentration. To prevent loss of vitE, feed samples were vacuum-packed and stored at -80 °C, and plasma samples were kept in plastic tubes at -20 °C until analysed. Samples were sent to the analysing laboratory using dry ice to assure the sample integrity during transport.

There was no reference tissue available, thus in-trial-samples were used as reference tissues.

Histological tissue preparation

Samples from the heart (right and left ventricles, septum), liver, and several skeletal muscles, including *Musculus* (*M*.) *semimembranosus*, *M*. *semitendinosus*, *M. long. dorsi*, *M. psoas major*, and *M. biceps brachii*, were fixed in a 4 % formaldehyde solution, embedded in paraffin, cut into 3-5 µm thick slices and stained with haematoxylin and eosin (HE) for evaluation (**Paper II**). The histopathological findings in skeletal muscles were graded blindly on a semi-quantitative scale of severity (0 - normal, 1 - mild changes, 2 - moderate changes, 3 - severe changes). The main difficulty here were the day-to-day differences in grading.

Statistical methods

All statistical analyses were performed using R (Version 1.1.383 – © 2009-2017 RStudio, Inc.) [302]. Linear model regression analysis (*lm*) includes both fixed and random effects and is particularly useful for the evaluation of repeated measurements.

Feed intake in sows (**Paper III**) was analysed using generalized additive models (GAM), which are useful when values obtained are non-linear. Feed intake in sows increases rapidly after farrowing and thus, linear regression is not applicable. GAM takes every independent variable (e.g., sow, diet) and fits functions to the corresponding measured/dependent variables (feed intake)

to minimize the deviation of the residuals and the number of degrees of freedom. The results from this analysis were used to narrow the values to curves to emphasize differences.

For gene expression results, absolute fold changes <1.5 were considered as random effects and were not discussed.

General discussion of main results

Symptoms caused by selenium (Se) deficiency are species-specific and in pigs commonly related to myopathies like Mulberry Heart Disease (MHD; myocardium) and nutritional myodegeneration (NMD), or to hepatopathy called *Hepatosis dietetica*. To obtain actualized data on MHD, a descriptive study was conducted on sudden-death cases of fast-growing, well-nourished pigs (**Paper I**). Results showed significantly different mean Se concentrations between MHD cases and controls in several tissues (myocardium, thymus, gastric ventricle, intestine, liver, kidney, nine of ten skeletal muscles analysed, and skin).

The breeding program pursued by Norsvin over the past decade has led to a reduction in the level of feed per kg of weight gain, and increased average daily gain (ADG) in weaned pigs and in grower-finishers as well as the lean meat percentage [303, 304]. Considering all these factors, one might conclude that the Se (and vitE) uptake of such pigs may not cover the nutritional requirements (**Paper I**). Specifically, the selenoprotein SelW is abundant in the myocardium and skeletal musculature in several mammalian species [305], where it is involved in muscle metabolism and prevents excessive oxidation [253]. Low expression levels of *SelenoW* in the skeletal muscle of pigs fed the unsupplemented control diet (**Paper II**) may indicate a low Se concentration in this tissue. Furthermore, this dependency of the *SelenoW* expression on the Se status (**Paper II**) might, at least partly, explain the occurrence of lesions in tissues with low Se concentrations such as myocardium and skeletal muscle tissue of MHD pigs (**Paper I**). The tissue distribution of SelW might relate to the species-specific pattern of lesions in Se deficiency syndromes [306, 307]. Speciation studies on muscle tissue from seven livestock species showed that SelW and GPx contained 85-100% of the recovered Se [308].

Another feature described by others is that the muscle fibre area supplied by one capillary is significantly larger in domestic pigs compared with wild boars [309]. This might result in a suboptimal supply of nutrients possibly reinforcing already existing impairment.

However, concerning porcine health, it is necessary to look at all the possible relations with the dietary Se level from the inorganic and organic Se sources provided and to align it with the actual Se requirement in high-yielding, lean pigs.

During the feeding trials presented herein (**Papers II-IV**), inorganic and organic Se sources were applied at and above the dietary maximum limits according to EU legislation as presented in the Figures 4 and 5. The studies aimed to evaluate Se-source- and -level-effects on performance parameters, haematology, clinical biochemistry, Se distribution, and Se speciation as well as expression of selected genes.

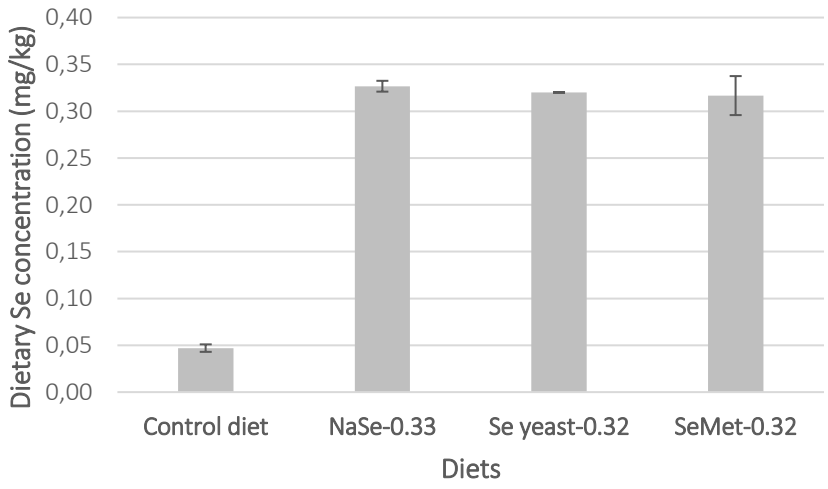

Figure 4 Dietary selenium concentrations throughout the grower-finisher trial *(mg Se/kg feed ±SD*; N=10/diet*;* ***Paper II****). Abbr.: NaSe – selenite-enriched, Se yeast – enriched with selenized yeast, SeMet – supplemented with L-selenomethionine*

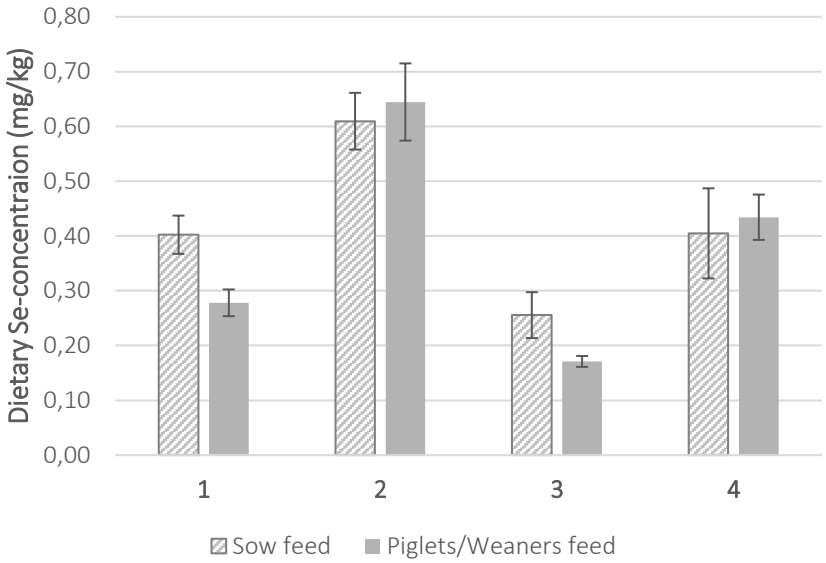

Figure 5 Dietary selenium concentrations throughout sows-and-offspring-trial *(mg Se/kg feed ±SD*; N=10/diet*; **Papers III and IV**). Diets 1 and 2: Selenite supplemented feed (1: selenite low, 2: selenite high). Diets 3 and 4: Feed enriched with L-selenomethionine (3: SeMet low, 4: SeMet high).*

Performance parameters

Feed intake in sows

The sows' feeding curve from farrowing and during lactation (**Paper III**) was generally in line with observations made in other studies [310, 311]. However, from day 13 of the lactation period, the sows fed SeMet-supplemented feed showed a significantly higher average daily feed intake compared with those fed selenite-enriched diets (**Paper III**). These results indicate that supplementing feed with SeMet during lactation could improve the sow's body Se reserves.

In Norway, the average lactation period is 33.3 d (2011-2020) [59, 60], which is longer as, e.g., in the USA with on average 28 days [312]. The higher feed consumption observed in SeMet-supplemented sows compared with those receiving dietary selenite over more than two weeks (**Paper III**) might have enabled them to maintain or re-establish their body condition for the

subsequent reproductive cycle. However, another study reported that a 4-weeks-lactation period is generally too short to compensate completely for the losses during the first weeks [312]. Also, Thingnes et al. [311] found no correlation between weight loss and feed intake during the last two weeks of lactation in Norwegian sows. Interestingly, the sows' feed intake in the present study was lower than that reported by Thingnes et al. [311], which might be due to the use of dry feed only in the trial presented herein (**Paper III**). The sows included in this study (**Paper III**) were accustomed to a liquid feeding system. For the actual study, however, it was necessary to dry-feed the sows during the experimental period because it was a field trial, and the feeding system did not allow for the distribution of different diets simultaneously.

Dietary composition and feed intake can directly affect the milk composition, especially the milk fat content [313]. However, feed intake is not a limiting factor for the milk yield of well-nourished sows [313]. Sows included in the present study (**Paper III**) received well-balanced diets and were fed following well-accepted Norwegian feeding regimes likely providing well-balanced amounts of nutrients according to international standards [214].

Weight gain suckling pigs and grower-finisher pigs

The studies on the offspring (**Paper IV**) of the above-mentioned sows (**Paper III**) showed that supplementation with SeMet at the dietary level of 0.26 mg Se/kg feed improved growth performance already during the suckling period until weaning compared with the piglets receiving milk from sows fed Se as selenite at a dietary level of 0.40 mg Se/ kg feed (day 24: 8.0 kg vs. 6.8 kg, and day 31: 10.1 kg vs. 8.5 kg BW; **Paper IV**). These results are in line with those obtained by Zhan et al. [314] and Cao et al. [315]. Besides, pigs heavier at weaning consumed less feed from weaning to 105 kg body weight than piglets with lower weaning weight [316]. Mahan et al. [316] showed that higher pre-weaning performance had a "carry-over" effect on the post-weaning performance until slaughter.

The Se-dependent deiodinases play key roles in the regulation of thyroid hormones and are responsible for the control of proper development, growth, and cell metabolism [105, 106]. Based on results on triiodothyronine (T3) and thyroxine (T4) concentrations in SeMet-fed pigs, Zhan et al. [237] suggested that both protein synthesis and energy production were improved, subsequently reflected in higher growth rates. Zhan et al. [237] found a positive correlation between antioxidant status and growth performance in SeMet-supplemented pigs, which is in line with our results on weight gain and Se status of piglets from SeMet-supplemented sows (**Paper IV**).

Influence of selenium level and source on haematological and clinical biochemical parameters as well as clinical symptoms

RBC-counts were lower in piglets at birth and 5 days of age in the SeMet-groups compared with piglets from sows fed NaSe-0.4 or NaSe-0.6 (**Paper IV**). These results might support findings made by Kaushal et al. [317] in mice highlighting the importance of adequate availability of Se in regulating red cell homeostasis. However, the availability of Se for selenoprotein synthesis might have been impaired in the SeMet receiving groups (**Paper IV**) due to the immaturity of the hepatic transselenation pathway (TS-pathway) in perinatal pigs [318, 319]. Se bound in Se species such as, for example, SeMet must be released by the TS-pathway, whereas Se from selenite is directly accessible to the selenoprotein synthesis. Subsequently, in piglets from SeMet-fed sows levels of selenoproteins might have been suboptimal for, among other things, erythropoiesis during the first days of life.

There were no clinical signs of Se deficiency in the control group in the grower-finisher trial, possibly due to the application of feed supplemented with 100 mg vitE/kg (**Paper II**). Se deficiency alone may not cause overt clinical injury, but it is shown to predispose animals to lesions caused by vitE deficiency and might deteriorate viral infections [320, 321]. However, none of the animals was Se-depleted before the feeding trials (**Papers II-IV**). As long as the pigs have Se resources like the selenoproteins GPx1 and SelW, or

SeMet, wherefrom Se can be released through the TS-pathway, its' buffer capacity can overcome short-term Se deficiencies especially in combination with sufficient amounts of synergetic antioxidants such as vitE. Several research groups have suggested a so-called 'Se cycle' in which some selenoproteins, e.g., GPx1 and SelW, serve as cellular "Se buffers", which are mobilized for the synthesis of other, more vital selenoproteins such as Dio1 under shortage conditions [322-326].

The influence of Se on some haematological parameters, such as RBC at birth (**Paper IV**), and neutrophils in farrowing sows (n.s.; **data not shown**) seemed to depend on both the dietary Se source and Se concentration as well as the production level and health status of the pig evaluated.

Selenium storage and transfer

Selenium intake in pigs and humans

The total Se intake in the sows (Figure 6) was significantly highest in those receiving NaSe-0.60 ($P<0.001$) throughout the trial.

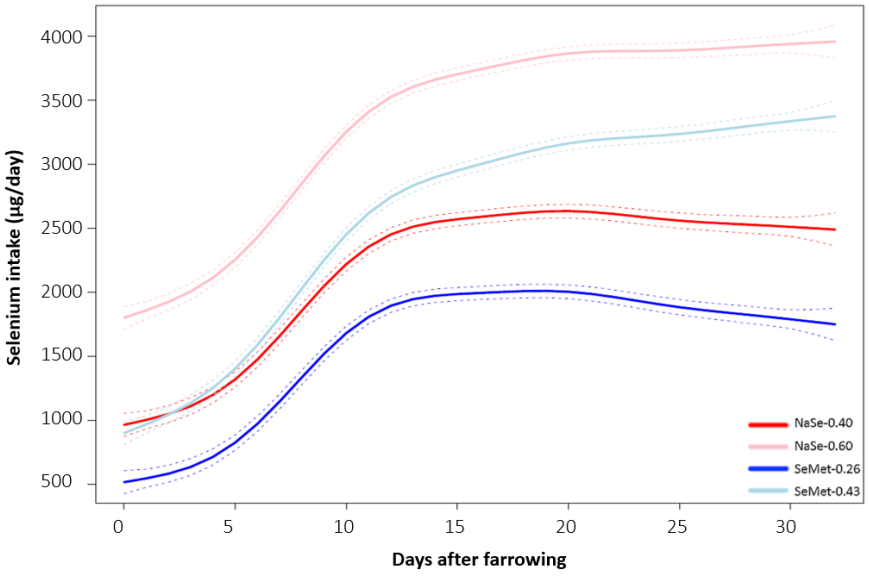

Figure 6 ***Selenium intake in lactating sows*** *calculated based on feed intake from farrowing to weaning (µg Se/day;* ***Paper III****)*

At farrowing, the Se intake was not significantly different in the three other feeding groups. This was neither reflected in the plasma Se concentrations of the neonatal piglets before colostrum intake (**Paper IV**), nor in the calculated colostral Se intake of the piglets (Table 10). The offspring from sows receiving SeMet had highest plasma Se concentrations, and, following the calculations based on the recommended minimum colostrum intake by Devillers et al. [327], their colostral Se intake was also highest.

***Table 10 Recommended human daily intake of selenium** (Se) [207] **versus daily intake in pigs throughout the present study** (in µg Se/day).*

Humans, physiological groups	**RDA^**	**Pigs, physiological groups**	**Group**	**N**	**Se intake in pigs**
Women, 60 kg	60	Sows, >200 kg	NaSe-0.40	7	1280.0±0.0
			NaSe-0.60	8	1920.0±0.0
			SeMet-0.26	7	832.0±0.0
			SeMet-0.43	8	1376.0±0.0
Lactating women	75	Lactating sows^^	NaSe-0.40	7	2560.0±0.0^{b*}
			NaSe-0.60	8	3840.0±0.0^{d*}
			SeMet-0.26	7	1901.7±84.0^{a*}
			SeMet-0.43	8	3246.5±109.9^{c*}
Infants (0 ≤ 4 months; breast-milk-fed)**	10	Neonatal piglets#	NaSe-0.40	7	32.6±2.2^{a*}
			NaSe-0.60	8	37.5±4.2^{a}
			SeMet-0.26	7	48.6±4.7^{ab}
			SeMet-0.43	8	58.3±4.6^{b}

^The saturation of SelP in plasma is used as a criterion for the derivation of reference values for Se intake in adults (µg/day) [207]; **is based on the Se concentration of breast milk, which is considered to be the optimal diet for infants [207]. ^^Results of Se intake presented are based on the feed intake on day 18 during lactation when most sows per group were fed the same amount of feed/day (**Paper III**; see also Figure 6). #Se intake calculated based on the recommended minimum colostrum intake of 200 g/d as published by Devillers et al. [327] (**Paper IV**). ##Data obtained from the offspring of the above-mentioned sows (**unpublished data**).
Means within a column without a common superscript differ significantly (P<0.0083). *P<0.001.

From day four of the lactation period throughout the trial, the Se intake in the sows was significantly lowest in SeMet-0.26 compared with all other groups ($P<0.001$).

Comparing Se intake in humans and pigs independent of age shows strikingly higher Se intakes in pigs (Table 10). This is in line with the statement from Penglase et al. [205] that the Se requirement of a species depends on the SeCys content in its SelP. The significant slower growth of humans compared with pigs and other animals [328], might decrease the requirement for selenoproteins. In addition, modern fast-growing livestock might have a higher requirement for antioxidants like Se as a consequence of selection for increased production efficiency. Modern Large White pigs showed considerably higher serum concentrations of ROS than wild boars [329]. A muscle fibre's ability to sustain physical stress is determined by its oxidative capacity, thus an increase in the glycolytic capacity due to selection for high lean growth rate may increase the animal's sensitivity to stressors [330].

Selenium transfer from sows to offspring

Statistically significant dose-dependent total Se concentrations were observed in milk from SeMet-supplemented sows, and tendential in their colostrum. This was not found in colostrum or milk from those fed selenite-supplemented diets (Paper III), which is in line with results from Mahan and Peters [202].

Furthermore, results from the present study (**Paper III** and Table 11) confirmed that colostrum contained much higher amounts of Se compared with milk [52, 331-333], which was mainly due to higher concentrations of SelP and SeAlb in colostrum (**Paper III**). Because tight junctions of the mammary gland are leaky until just prior to parturition [334], these plasma proteins can flow freely between cells and spill back into the blood.

The concentration of SelP decreased more than that of SeAlb from colostrum to mature milk collected at weaning (**Paper III**). SeAlb was similarly detected

in colostrum and milk of all sows independent of the Se source applied (**Paper III**).

Table 11 ***The Se concentrations in plasma, colostrum and milk, and the resulting colostrum- or milk-Se/plasma-Se ratio in sows*** *(Mean ± SD).*

Parameter[1]	**Initiation of the trial**	**Farrowing**	**Weaning**
Plasma Se (mg/kg)			
NaSe-0.40	0.16±0.03[a]	0.18±0.03[a]	0.24±0.05[a]
NaSe-0.60	0.21±0.03[a]	0.18±0.04[a]	0.24±0.04[a]
SeMet-0.26	0.18±0.04[a]	0.19±0.02[a]	0.26±0.03[a]
SeMet-0.43	0.18±0.03[a]	0.20±0.04[a]	0.28±0.02[a]
Se in colostrum/milk (mg/kg)		**Se in colostrum**	**Se in milk**
NaSe-0.40		0.16±0.03[a]	0.04±0.00[a]
NaSe-0.60		0.19±0.06[ab]	0.04±0.01[a]
SeMet-0.26		0.24±0.06[bc]	0.07±0.00[b*]
SeMet-0.43		0.29±0.06[c]	0.12±0.01[c*]
Ratio		**Colostrum/plasma-Se**	**Milk/plasma-Se**
NaSe-0.40		0.93±0.20[a]	0.16±0.04[a]
NaSe-0.60		1.26±0.30[ab]	0.15±0.05[a]
SeMet-0.26		1.40±0.27[ab]	0.28±0.04[b]
SeMet-0.43		1.59±0.52[b]	0.43±0.02[c]

Means within a column with different superscripts differ significantly ($P < 0.05$). *$P < 0.001$.

Also, Mou et al. [335] detected higher SelP- concentrations in colostrum compared with milk independent of the Se source applied. The sows in the present study received a Se yeast-supplemented diet until entering the trial, and thus, SeMet might have been stored in the body tissues (**Paper III**). The maternal protein turnover increases during gestation and releases amino acids from maternal body proteins [10]. A release of the selenized proteinogenic amino acid SeMet from maternal body protein might explain, at least partly, the occurrence of SeAlb in colostrum and milk from sows of all diet groups (**Paper III**).

The colostrum/plasma- and the milk/plasma-ratio for total Se demonstrated a higher transfer of Se from plasma to milk in sows supplemented with SeMet than in sows supplemented with selenite (Table 11 and **Paper III**), which was in line with results from Zhan et al. [237]. In conclusion, the higher Se concentration in colostrum and milk from SeMet-supplemented sows could lead to an elevated antioxidant capacity in suckling piglets since significantly higher plasma levels of the antioxidant selenoprotein GPx3 were found in neonatal piglets from these sows (**Paper IV**).

Selenoproteins in porcine plasma

In plasma, Se is mainly bound to the selenoproteins SelP and GPx3 [336]. Only SelP was measured in the sows' plasma at farrowing and weaning, colostrum and milk (**Paper III**). There was a trend towards higher concentrations of SelP in the sows' plasma at weaning (**Paper III**).

SelP is synthesized in the liver and released into the bloodstream [337]. Kasik and Rice [338] detected increased *SelenoP*-mRNA levels in the liver of pregnant mice, possibly representing a mechanism to facilitate and enhance storage and transport of Se. At farrowing, porcine maternal plasma SelP levels were lower compared with weaning (**Paper III**). Following increased levels of *SelenoP* expression in the maternal liver during pregnancy [338], one should expect higher levels of this Se transport protein in maternal plasma at the end of gestation. There are at least two possible explanations for this phenomenon: Firstly, during late gestation, the blood volume increases leading to haemodilution [339]. Secondly, SelP was shown to be the major Se transport protein to the murine foetus [340].
Kasik and Rice [338] showed decreasing mRNA levels in maternal mouse liver to non-pregnant levels within 7 d postpartum, which might explain lower SelP levels observed in sows plasma at weaning (**Paper III**).

Lower plasma SelP concentrations were observed in neonatal versus 5-d-old piglets (**Paper IV**), which was in line with Mou et al. [335]. Besides, the relative fraction (%) of Se associated with SelP was higher in piglets' plasma

of all feeding groups at birth and 5-days of age compared with that in the corresponding sows at farrowing (**Paper IV**). Moreover, plasma SelP levels increased stronger during the first 5 days of life in piglets originating from SeMet-fed sows compared with those from sows supplemented with selenite (**Paper IV**).

SelenoP mRNA was not detectable in foetal mouse liver on day 5 before birth, but its expression increased starting 4 d before birth reaching adult levels by the time of birth [338]. Furthermore, the activity of the hepatic TS-pathway, giving access to Se bound in Se species such as SeMet, increases rapidly postnatal in piglets [319]. In addition to the transport protein function, SelP is involved in defending the organism against the damaging effect of free radicals and is a good indicator of Se resources in the organism [172].

High levels of GPx3 were detected in the plasma of neonatal piglets, especially in the offspring from SeMet-fed sows (**Paper IV**). GPx3 is produced in the kidney and, for the offspring from SeMet-fed sows, it reflects a high capacity of the TS-pathway in the perinatal porcine kidneys [341]. At 5 d of age, the GPx3 level was significantly lower in all groups, but still higher in the feeding groups receiving SeMet (**Paper IV**).

Based on the results presented in **Paper IV** it is suggested that piglets from SeMet-fed sows have a better antioxidant status compared with those from selenite-fed sows. Furthermore, the need for SelP as an antioxidant and its utility as an indicator of Se status might be age dependent.

Selenium retention in tissues

In this study, Se deposition from SeMet occurred mainly in the skeletal muscles, myocardium from neonatal piglets and grower-finisher pigs and in tendency also in the testes from 14-day old pigs (**Paper IV**), which is in line with results from a study in rats [239]. Dietary SeMet was superior to selenite and Se yeast in that it led to higher tissue concentrations of Se, especially muscle tissue (**Papers II** and **IV**). Se yeast contains a mixture of Se species

including a certain fraction of SeMet [342], but only SeMet can be, in place of methionine, incorporated in the amino acid chain of body proteins and thus retained.

Supplementation with SeMet above legal EU limits in piglets resulted in even higher retention of Se in various tissues, such as testes, skeletal and cardiac muscles compared with supplementation of a somewhat lower level of SeMet, or selenite at any dietary Se concentration (**Paper IV**). This might be positive in terms of maintaining a stable Se status in the pig and non-vegetarian humans, especially in populations with suboptimal or even deficient Se status.

As opposed to grower-finisher pigs (**Paper II**), the mean liver Se concentration in neonatal piglets was not significantly influenced by Se source, and the kidney Se concentration was lower in piglets (**Paper IV**) than in grower-finisher pigs (**Paper II**).

Nakagaki et al. [343] showed that the hepatic metabolic activity reached full capacity three to four weeks after birth in mice and humans. Rising renal Se concentrations during growth reflected the kidneys' function in Se retention and excretion via the urine [344]. The kidney is rich in Se because of the synthesis of GPx3 [341] and its function in preventing the loss or degradation of SelP, which was shown to be specifically bound by the receptor megalin in the kidney of rodents [345].

Liver, kidney, and testes are shown to be rich in SeCys. Thus, neonatal Se concentrations in the liver and kidney might be a sign of the immaturity of enzymatic pathways. In addition, the testicular Se concentration is shown to be age dependent, increasing during puberty [346, 347]. Also, Dalto et al. [348] suspected the involvement of age and physiological stage in the Se-source response of organs in pigs.

Gene expression

Analysis of the expression of selected seleno- and non-selenogenes (**Paper II**) (format: *italic*) was conducted in whole blood samples isolated before and

after LPS-injection. In addition, during the grower-finisher period, the expression of a slightly different panel of genes was analysed in samples isolated from the long back muscle *Musculus (M.) longissimus dorsi*. Selenogenes (*gpx1*, *gpx3*, *SelenoN*, *SelenoS*, *txnrd1*) code for selenoproteins (GPx1, GPx3, SelN, SelS, Txnrd1) whereas non-selenogenes (e.g., cyclooxygenase-2 - *cox2*, inducible nitric oxide synthase - *inos*, catalase - *cat*) included in this section encode endogenous antioxidant enzymes (e.g., *cat* - Cat) and cytokines (e.g., interferon-gamma, *ifnɣ* - IFN-ɣ) and other immune response-related proteins (e.g., *cox2* –Cox2 and *inos* - iNOS). Additionally, the gene expression of the non-selenogenes F-Box protein 32 (*Fbxo32*) and caspase 3 (*casp3*) was analysed in samples from *M. long. dorsi*.

Gene expression studies on whole blood

The gene expression studies on whole blood isolated prior to the LPS challenge revealed that *txnrd1*, *SelenoN*, and *SelenoS* as well as *cat*, were expressed to a greater extent in pigs fed selenite compared with pigs in the other feeding groups (**Paper II**).

The products of all these genes are intertwined with the antioxidant system. Txnrd1 is considered a key enzyme involved in selenite metabolism [349] and its activity is increased by selenite [350]. Metabolizing the rather strong oxidizing selenite can trigger ER stress due to the generation of ROS [351, 352]. SelN and SelS, an endoplasmatic reticulum (ER) stress response protein [353], are transmembrane proteins localized to the ER, involved in the calcium (Ca^{2+}) homeostasis and regulation of ER stress [354, 355]. The antioxidant enzyme Cat, found in all aerobic organisms, catalyses the decomposition of H_2O_2 to oxygen and water [61]. As mentioned above, selenite metabolism can increase ROS production, which in turn may have increased the requirement for the enzyme Cat to prevent oxidative damage (**Paper II**).

A stronger downregulation of *SelenoN* after LPS application was observed in pigs fed NaSe-0.3 and NaSe-0.6 (**Paper II**) and might influence its function in

the regulation of the Ca^{2+} homeostasis in the cell [356]. This possibly leads to a disturbance of the neutrophilic migratory behaviour (**Paper II**).

In whole blood, the observed upregulation of *SelenoK* as well as the downregulation of *SelenoN*, *cox2*, and *inos* in the first h after the LPS application might be important prerequisites to enable transendothelial migration of leucocytes (**Papers II**). SelK has been shown to be required for Ca^{2+} flux in immune cells, involved in T-cell proliferation as well as T-cell and neutrophil migration [127-129].

Gene expression studies in *M. longissimus dorsi*

The expression of the *SelenoW* gene in this skeletal muscle was Se-dependent resulting in lower mRNA levels in the control animals fed a diet low in Se (**Paper II**). In contrast to e.g., *gpx1*, tissue-specific and Se-dependent modulation of *SelenoW* gene expression has been shown by others and might relate to its most important sites of function in Se deficiency and excess [306, 357-361]. SelW is important in muscle tissue metabolism [253, 305]. In chicken muscle, SelW knockout has been shown by others to induce Ca^{2+} leak, oxidative stress, and Ca^{2+} channel reduction [362]. Several other selenoproteins are also involved in the Ca^{2+} metabolism of cells and are shown to depend on the Se status.

Taken together, dietary Se sources as well as the pigs' Se status affect the expression of selenogenes and non-selenogenes in whole blood and muscle tissue. Moreover, since lower feed intake is a part of sickness behaviour, a combination of inorganic (selenite) and organic (e.g., SeMet) Se sources in pig feed might be advantageous to support selenoprotein expression and a stable Se status during periods of suboptimal Se intake.

Relationships with other nutrients including molybdenum and magnesium

Shortly after LPS-application, an increase of plasma-molybdenum (Mo)-concentrations was observed in the Se-supplemented groups of pigs in the grower-finisher trial (**Paper II**). Mo-containing enzymes are involved in the

turnover of Se compounds and selenoproteins for the liberation of Se via the TS-pathway [363]. LPS has been shown to increase protein degradation [364] which might lead to release of protein-bound SeMet.

In the grower-finisher trial, the lowest plasma magnesium (Mg) levels were found in the pigs receiving the unsupplemented diet and in those receiving selenite at a dose of 0.3 mg Se/kg (**Paper II**). Mg is essential for the synthesis of GSH [365, 366] the major thiol antioxidant. Hepatic GSH synthesis and release into the bloodstream increased significantly in Se-deficient rats, suggesting it might be a phenomenon in Se deficiency [367]. GSH is essential in the erythrocytic selenite metabolism [96]. Thus, higher plasma Mg in pigs supplemented with organic Se (Se yeast or SeMet; **Paper II**) might indicate an improved antioxidant capacity.

Vitamin E and other antioxidants

All trial diets were enriched with 100 mg vitE/kg diet (**Papers II-IV**). Data obtained from piglets (**Paper IV** and Table 12) showed low plasma concentrations of major antioxidants such as vitE, which is in line with results from studies in pigs [368], and ceruloplasmin as also shown in human neonates [48]. Plasma vitE levels in the study presented herein (Table 12 and **Paper IV**) decreased again after weaning, as is shown by others [30, 197, 369]. Additionally and corroborating previous studies [30], this coincided with an increase of Se levels (**Paper IV**). It is suggested that levels of Se and vitE partially compensate for each other during the weaning period [370].

Table 12 Clinical biochemistry and vitE measurements from birth until 38 days of age (Mean ± SEM).

	NaSe-0.4	NaSe-0.6	SeMet-0.2	SeMet-0.4
At birth				
VitE*	0.31±0.02[a]	0.24±0.02[a]	0.31±0.01[a]	0.31±0.03[a]
Cu (µmol/L)	3.8±0.2[a]	3.6±0.3[a]	3.2±0.4[a]	3.9±0.4[a]
Cp (mg/dL)	9.8±0.6[a]	8.0±1.3[a]	9.4±0.7[a]	8.2±0.4[a]
Cp/Cu	*no paired samples*			
Bilirubin	4.1±0.6[a]	3.3±0.7[a]	4.3±0.9[a]	3.4±0.6[a]
day 5				
VitE*	5.48±0.57[a]	5.69±0.48[a]	4.50±0.34[a]	4.50±0.31[a]
Cu (µmol/l)	19.3±1.0[a]	19.7±0.9[a]	23.7±1.1[b]	20.5±0.7[ab]
Cp (mg/dL)	40.9±4.2[a]	42.8±4.1[a]	62.9±5.5[b]	42.1±3.1[a]
Cp/Cu	2.1±0.1[a]	2.1±0.1[a]	2.6±0.1[a]	2.1±0.2[a]
Bilirubin	4.0±0.4[a]	3.1±0.6[a]	2.9±0.5[a]	3.2±0.5[a]
day 24				
VitE*	2.08±0.10[a]	1.84±0.11[a]	1.99±0.33[a]	1.24±0.17[a]
Cu (µmol/l)	28.7±1.0[a]	28.2±0.8[a]	29.5±0.7[a]	27.9±0.6[a]
Cp (mg/dL)	90.7±6.1[ab]	96.1±4.9[b]	91.7±5.1[b]	70.2±4.2[a]
Cp/Cu	3.2±0.2[b]	3.4±0.1[b*]	3.1±0.1[b]	2.5±0.1[a]
Bilirubin	7.5±0.9[b]	5.2±0.7[ab]	4.0±0.6[a]	3.6±0.4[a*]
day 38				
VitE*	1.06±0.11[a]	0.80±0.05[a]	0.95±0.09[a]	0.75±0.12[a]
Cu (µmol/l)	24.5±1.0[a]	24.0±1.6[a]	26.3±0.5[a]	26.7±1.8[a]
Cp (mg/dL)	90.6±7.6[a]	91.1±12.1[a]	97.9±4.2[a]	109.5±11.9[a]
Cp/Cu	3.7±0.2[a]	3.8±0.2[a]	3.7±0.1[a]	4.0±0.2[a]
Bilirubin	1.6±0.3[a]	1.5±0.2[a]	1.5±0.2[a]	1.4±0.2[a]

*Values from VitE (α-tocopherol; µg/mL) analysis are presented with two decimals due to low values. **Due to technical problems. <LOD: Underneath the lower limit of detection. Means (n=7) within a row without a common superscript differ significantly (P<0.0083). *P<0.001.

Newly weaned pigs have to cope with many stressors including mixing with unfamiliar animals, new solid diets that could trigger neophobia, transportation, new environments, and maternal separation [371]. In this critical period, many weaned pigs are reluctant to eat, leading to weight loss during the first days after weaning [372, 373]. The coincidence of all these events might decrease the overall capacity of the antioxidant system and subsequently play a role in the increased occurrence of MHD at that age [40, 192, 200, 374]. The pigs in the grower-finisher trial were fed the trial diets beginning at 11 weeks of age (**Paper II**), and at that age vitE deficiency was not expected to occur [197].

From the age of 24 d onward, RBC exceeded RBC counts published by Thorn [375] (BW in 20-day-old piglets: 4.76 kg) (**Paper IV**). These values are not consistent with the fast-growing pigs used in this study (**Paper IV**) as it was shown by others that haematological status and growth performance are positively correlated in pigs [376].

Rootwelt et al. [377] showed that piglets with a higher Hgb at birth (98 g/L [n=215] versus 86 g/L [n=47]) had a better chance to survive the suckling period. Landrace x Yorkshire Z-line sows (ZL, TN 70; Topigs Norsvin) are selected to produce large litters with uniform and heavy piglets with high viability [7]. Higher RBC-counts and Hgb concentrations may be necessary to provide the rapidly growing cells with sufficient amounts of oxygen. Moreover, higher Hgb concentrations might improve the defence against ROS produced during fast growth (**Paper IV**).

Erythrocytes are the major cellular component of blood, and erythrocyte membrane fluidity can be affected by oxidative stress [378]. Thus, the higher the count of needy cells, the lower the plasma VitE-concentration. In conclusion, high-yielding pigs might have a higher vitE requirement than slower growing hybrids.

Limitations

- The lack of a non-supplemented control group in the sows-and-offspring trial was an experimental limitation. However, the nutritional requirements of the high prolific animals had to be covered to avoid production losses.
- Sows in the sows-and-offspring trial were not weighed as a result of limited personnel. Weighing the sows throughout the study would have shown whether the increased feed intake in SeMet-supplemented sows influenced their BW.
- The low number of individuals included in both the grower-finisher trial and in the sows-part of the sows-and-offspring trial limited the statistical power of the study.
- The animals were not Se-depleted before the feeding trials and the sows had received feed enriched with an organic Se source (Se yeast). The effect of previous Se supply might have influenced results of the total Se measurements, gene expression, and Se speciation as well as the occurrence of Se-and-vitE-responsive conditions.
- Trial diets were enriched with a rather high vitE level (100 mg vitE/kg feed). This may have restricted eventual signs of impairment of the health status, gene expression, and Se speciation (**Papers II-IV**).
- Results of Se speciation comprised only about 34 % of total Se in colostrum and 13 % in milk at weaning. GPx3 was unfortunately not determined in the sows' plasma, colostrum nor in milk due to the lack of methodological requirements.
- Se speciation methods were not developed when the first LPS study was performed (Paper II).
- More effects variables (ROS, micronuclei, DNA damage etc.) should have been included.

Future research questions

- In the case of Se-and-vitE-responsive conditions, several studies determining the total amount of Se have been conducted. Today, it seems insufficient to simply examine variation in total element levels as an indicator of disease states and pathogenesis. Since Se exerts its function via selenoproteins, Se speciation should be applied on different tissues to get closer to the cause of the different clinical manifestations of vitE/Se deficiency.
- Speciation results presented herein covered only about 80% of the total Se concentration depending on the material analysed (plasma, colostrum, milk). It would therefore be of interest to clarify a) the other Se species to which Se is bound in porcine plasma, colostrum and milk and b) if there are any significant differences caused by the Se source fed and/or the pigs' age.
- SelP has been accepted as an indicator of the Se status and functions as Se transport protein and antioxidant. Is the significance of SelP as an antioxidant and its usability as an indicator of the individual's Se status age dependent?
- Selenium is shown to positively influence fertility in males, e.g., mice and humans. However, little research has been conducted on fertility in boars.
- Inclusion of higher amounts of vitE in diet has been shown to increase the metabolism of vitE. Are there any differences between lean and fat pig hybrids?
- Do the amount and distribution of vitE in porcine tissues differ between lean and fat pig hybrids? Might this influence the vitE depletion, Se metabolism, or Se speciation? Is there a link to the re-emergence of Se-and-vitE-responsive conditions?
- Do modern, fast-growing pigs have a higher vitE requirement due to higher numbers of erythrocytes compared with slower growing and/or less lean hybrids?

Conclusions and implications

To the best of the researchers' knowledge, the research group was the first to report on the concentrations of the Se species SelP, SeAlb, and SeMet in body fluids from sows (plasma, colostrum, and milk; **Paper III**) as well as on these Se species and GPx3 in piglet plasma (**Paper IV**).

The different applied Se sources influenced Se speciation, total Se concentrations in some selected tissues, gene expression of seleno- and non-selenogenes as well as body weight, feed intake, and some haematological and clinical-biochemical parameters in pigs.

Se speciation results depended on the material analysed, Se source, dietary Se level, and the individual's age (**Papers III and IV**). Results pertaining to total Se and its speciation in plasma, as well as RBC and growth performance obtained from the pigs during the suckling period showed that a combination of inorganic and organic Se seemed beneficial. Se speciation results from sows and their offspring depended on the material analysed, Se source, dietary Se level, and the individual's age. Gene expression was influenced not only by Se source and dietary level but also by the sample examined (blood vs. skeletal muscle). Furthermore, the maturity of the transselenation pathway has to be considered, depending on the Se species and material evaluated when analysing Se source effects on foetal and suckling pigs. Lower levels of oxidative stress may have re-directed energy costs to performance as indicated by higher ADG in SeMet-0.2-fed pigs compared to selenite-fed pigs or those receiving SeMet at a higher dietary level. Results concerning gene expression, haematology and biochemistry from the presented feeding trials also indicated that selenite supplementation at close-to-allowed concentrations increases oxidative stress levels in the modern fast-growing, feed-efficient pigs. The impact of this increase depended on the level of physiological and management-related stress experienced by the animal sampled. In addition, the effects of selenite on haematology seemed to depend on age and occurrence of an additional stress factor, e.g., LPS. However,

selenite in the diet of gestating sows seemed to be favourable in terms of foetal haematopoiesis.
Since Se (and vitE) concentrations in skeletal muscle tissues are more stable over a longer period than in the liver, for example, these tissues should be included in the MHD-sampling panel.
Due to higher RBC, modern fast-growing pigs might have increased vitE requirements compared with former hybrids. This study confirmed the superiority of SeMet versus selenite with regard to tissue retention and transfer from sows to their offspring. Se deposition depended on the Se source, tissue, and age of the pig with SeMet giving the highest Se concentrations.

In conclusion, only SeMet seemed to meet the grower-finisher pigs' requirement when used at the actual EU-mandated legal limits for dietary Se and confirmed the Se requirement as predicted by the SeCys content of the porcine SelP as an indicator for the Se requirement in pigs. However, it seems advantageous to combine organic and inorganic Se sources in porcine diets, e.g., 0.2 mg/ Se from the organic Se source SeMet and 0.2 mg Se/kg feed from selenite, when supplementing pig feed based on Norwegian grain low in Se. The resulting dietary Se concentration complies with the current EU-legislation.

References

1. Mahan, D.C., *Nutrition and management of high-producing swine*. 2012, OHIO STATE UNIVERSITY, USA: https://portal.nifa.usda.gov/web/crisprojectpages/0136430-nutrition-and-management-of-high-producing-swine.html.
2. Thingnes, S.L., et al., *The effect of different dietary energy levels during rearing and mid-gestation on gilt performance and culling rate.* Livest Sci, 2015. **172**: p. 33-42.
3. Holm, B., et al., *Genetic correlations between reproduction and production traits in swine.* J Anim Sci, 2004. **82**(12): p. 3458-3464.
4. Merks, J.W., P.K. Mathur, and E.F. Knol, *New phenotypes for new breeding goals in pigs.* Animal, 2012. **6**(4): p. 535-543.
5. Ball, R.O., R.S. Samuel, and S. Moehn, *Nutrient requirements of prolific sows*, in *Banff Pork Seminar 2008. Changing the Industry with New Solutions.* 2008, Department of Agricultural, Food and Nutritional Science, University of Alberta: Banff, Alberta, Canada. p. 223-237.
6. Norsvin, T. *Avlsmål Duroc [Breeding goals Duroc]*. 2012 [cited 2017 23.05.2017].
7. Topigs Norsvin *Topigs Norsvin LZ 70*. The Insider, 2014. **Fall 2014**, 5.
8. Topigs Norsvin *Norsvin Landrace*. The Insider, 2014. **Winter 2014**, 4-5.
9. Noblet, J., et al., *Metabolic utilization of energy and maintenance requirements in growing pigs: effects of sex and genotype.* J. Anim. Sci., 1999. **77**(5): p. 1208-1216.
10. Thingnes, S.L., et al., *The effect of different dietary energy levels during rearing and mid-gestation on sow lifetime performance and longevity.* Acta Agric Scand A Anim Sci, 2015. **65**(3-4): p. 148-157.
11. Prunier, A., et al., *Productivity and longevity of weaned sows*, in *Weaning the pig - concepts and consequences*. 2003, Wageningen Academic Publishers.
12. Zhang, Y., et al., *Increased consumption of sulfur amino acids by both sows and piglets enhances the ability of the progeny to adverse effects induced by lipopolysaccharide.* Animals (Basel), 2019. **9**(12): p. 1048-1061.
13. Merks, J.W.M. *One century of genetic changes in pigs and the future needs.* in *The Challenge of Genetic Change in Animal Production.* 2000. Edinburgh, Scottlandd: BSAS (2000) 86/pp.
14. Hribar, C., *Understanding Concentrated Animal Feeding Operations and Their Impact on Communities*, M. Schultz, Editor. 2010, National Association of Local Boards of Health: Ohio, USA.
15. Oksbjerg, N., et al., *Long-term changes in performance and meat quality of Danish Landrace pigs: a study on a current compared with an unimproved genotype.* Anim Sci, 2000. **71**(1): p. 81-92.

16. Wetten, M., et al., *Simultaneous estimation of daily weight and feed intake curves for growing pigs by random regression.* Animal, 2011. **6**(3): p. 433-439.
17. Pew Commission on Industrial Farm Animal Production, *Putting meat on the table: Industrial farm animal production in America*. 2008: http://www.pewtrusts.org/~/media/legacy/uploadedfiles/peg/publications/report/pcifapfinalpdf.pdf.
18. Prunier, A., M. Heinonen, and H. Quesnel, *High physiological demands in intensively raised pigs: impact on health and welfare.* Animal, 2010. **4**(6): p. 886-898.
19. van der Waaij, E.H., *A resource allocation model describing consequences of artificial selection under metabolic stress.* J Anim Sci, 2004. **82**(4): p. 973-981.
20. Kalmar, I.D., D. Vanrompay, and G.P.J. Janssens, *Broiler ascites syndrome: Collateral damage from efficient feed to meat conversion.* The Veterinary Journal, 2013. **197**(2): p. 169-174.
21. European Commision, *Commission Directive 2001/93/EC of 9 November 2001 amending Directive 91/630/EEC laying down minimum standards for the protection of pigs*. 2001.
22. Mattilsynet [Norwegian Food Safety Authority], *Forskrift om hold av svin. [Pig husbandry]*, in *FOR-2003-02-18-175*. 2020: https://lovdata.no/dokument/SF/forskrift/2003-02-18-175.
23. Norwegian Meat and Poultry Research Center, *Kjøttets tilstand - Status i norsk kjøtt- og eggproduksjon [Report on the status of the Norwegian meat and egg production]*. 2017: http://flashbook.no/animalia/kjottetstilstand16/pubData/source/161684-Animalia-KT16-13-DSA.pdf.
24. Norwegian Meat and Poultry Research Center, *Helsetjenesten for svin - Årsrapport 2015 [Pig Health Services - Annual report 2015]*. 2016: http://www.animalia.no/upload/FIler%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport%202015.pdf.
25. Jørgensen, H.J., et al., *The Norwegian Zoonoses Report 2015*, N.V. Institute, Editor. 2016, Norwegian Veterinary Institute: http://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2016/norwegian-zoonoses-report-2015.
26. Azerpajouh, S., et al. *Feed efficient pigs deal better with stress*. 2016. DOI: http://www.pigprogress.net/Sows/Articles/2016/9/Feed-efficient-pigs-deal-better-with-stress-2868050W/.
27. The Council of the European Communities, *COUNCIL DIRECTIVE of 23 November 1970 concerning additives in feeding-stuffs (70/524/EEC).* OJEC, 1970(L 270/1).
28. Agency for Toxic Substances and Disease Registry, *Toxicological profile for selenium*. 2003: Georgia, USA. p. 23-215.

29. Oropeza-Moe, M., H. Wisloff, and A. Bernhoft, *Selenium deficiency associated porcine and human cardiomyopathies.* J Trace Elem Med Biol, 2015. **31**: p. 148-156.
30. Sivertsen, T., et al., *Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds.* Acta Vet Scand, 2007. **49**: p. 1-9.
31. Lushchak, V.I., *Free radicals, reactive oxygen species, oxidative stress and its classification.* Chem Biol Interact, 2014. **224C**: p. 164-175.
32. Romero, A.C., et al., *The exogenous antioxidants*, in *Oxidative stress and chronic degenerative diseases - A role for antioxidants*, J.A. Morales-Gonzalez, Editor. 2013, InTech. p. 33-57.
33. Peternelj, T.-T. and J.S. Coombes, *Antioxidant supplementation during exercise training.* Sports Med, 2011. **41**(12): p. 1043-1069.
34. Gaté, L., et al., *Oxidative stress induced in pathologies: The role of antioxidants.* Biomed Pharmacother, 1999. **53**(4): p. 169-180.
35. Van Metre, D.C. and R.J. Callan, *Selenium and vitamin E.* Vet Clin North Am Food Anim Pract, 2001. **17**(2): p. 373-402.
36. Duarte, T.L. and J. Lunec, *Review: When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C.* Free Radic Res, 2005. **39**(7): p. 671-686.
37. Diplock, A.T., et al., *The nature of the acid-volatile selenium in the liver of the male rat.* Biochem J, 1973. **134**(1): p. 283-293.
38. Mertz, W., *Metabolism and Metabolic Effects of Trace Elements*, in *Trace elements in nutrition of children*, R.K. Chandra, Editor. 1985, Nestlé Nutrition; Raven Press: Vevey Switzerland. p. 107-119.
39. Hidiroglou, N., et al., *Comparative vitamin E requirements and metabolism in livestock.* Ann Rech Vet, 1992. **23**(4): p. 337-59.
40. Done, S., S.M. Williamson, and B.W. Strugnell, *Nervous and Locomotor Systems.*, in *Diseases of Swine. 10th Edition*, J.J. Zimmerman, et al., Editors. 2012, John Wiley and Sons Ltd.: UK. p. 294-328.
41. Munsterhjelm, C., et al., *Housing during early pregnancy affects fertility and behaviour sows.* Reprod. Dom. Anim., 2008. **43**(5): p. 584-591.
42. Mendl, M., A.J. Zanella, and D.M. Broom, *Physiological and reproductive correlates of behavioural strategies in female domestic pigs.* Anim Behav, 1992. **44**(6): p. 1107-1121.
43. Edwards, R.L., et al., *Reproductive performance of gilts following heat stress prior to breeding and in early gestation.* J Anim Sci, 1968. **27**(6): p. 1634-1637.
44. Omtvedt, I.T., et al., *Influence of heat stress during early, mid and late pregnancy of gilts.* J Anim Sci, 1971. **32**(2): p. 312-317.
45. Tsuma, V.T., et al., *Cortisol and β-endorphin levels in peripheral circulation around weaning in primiparous sows.* Anim. Reprod. Sci., 1995. **37**(2): p. 175-182.

46. Berchieri-Ronchi, C.B., et al., *Oxidative stress status of highly prolific sows during gestation and lactation.* Animal, 2011. **5**(11): p. 1774-1779.
47. Cottrell, E.C. and J.R. Seckl, *Prenatal stress, glucocorticoids and the programming of adult disease.* Front. Behav. Neurosci., 2009. **3**: p. 1-9.
48. Gitto, E., et al., *Oxidative stress in resuscitation and in ventilation of newborns.* Eur Respir J, 2009. **34**(6): p. 1461-1469.
49. Yin, J., et al., *Birth oxidative stress and the development of an antioxidant system in newborn piglets.* Free Radic Res, 2013. **47**(12): p. 1027-1035.
50. Saugstad, O.D., *Oxidative stress in the newborn--a 30-year perspective.* Biol Neonate, 2005. **88**(3): p. 228-236.
51. Morton, S.U. and D. Brodsky, *Fetal physiology and the transition to extrauterine life.* Clin Perinatol, 2016. **43**(3): p. 395-407.
52. Przybylska, J., E. Albera, and M. Kankofer, *Antioxidants in bovine colostrum.* Reprod Domest Anim, 2007. **42**(4): p. 402-9.
53. Halliwell, B. and J.M. Gutteridge, *Oxygen toxicity, oxygen radicals, transition metals and disease.* Biochem J, 1984. **219**(1): p. 1-14.
54. Barbusinski, K., *Fenton reaction - Controversy concerning the chemistry*. Vol. 16. 2009. 347-358.
55. Colson, V., et al., *Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses.* Appl Anim Behav Sci, 2006. **98**(1): p. 70-88.
56. Hampson, D.J., *Alterations in piglet small intestinal structure at weaning.* Res Vet Sci, 1986. **40**(1): p. 32-40.
57. Cera, K.R., et al., *Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine.* J Anim Sci, 1988. **66**(2): p. 574-584.
58. Weary, D.M., J. Jasper, and M.J. Hötzel, *Understanding weaning distress.* Appl Anim Behav Sci, 2008. **110**(1): p. 24-41.
59. Ingris, *Årsstatistikk 2015 (Annual statitics 2015)*. 2016: http://www.animalia.no/upload/Ingris%20web/Ingris_A%cc%8arsstatistikk_2015.pdf.
60. Ingris, *Ingris Årsstatistikk 2020 [Ingris Annual statitics 2020]*. 2021: https://www.animalia.no/globalassets/ingris---dokumenter/arsstatistikk-2020-007---endelig.pdf.
61. Zhang, Z., et al., *Meta-analysis of the correlation between selenium and incidence of hepatocellular carcinoma.* Oncotarget, 2016. **7**(47): p. 77110-77116.
62. Zhang, X., et al., *Selenium status and cardiovascular diseases: meta-analysis of prospective observational studies and randomized controlled trials.* Eur J Clin Nutr, 2016. **70**(2): p. 162-169.
63. Babaknejad, N., et al., *The relationship between selenium levels and breast cancer: a systematic review and meta-analysis.* Biol Trace Element Res, 2014. **159**(1): p. 1-7.

64. He, D., et al., *Serum selenium levels and cervical cancer: systematic review and meta-analysis.* Biol Trace Elem Res, 2017. **179**(2): p. 195-202.
65. Pinsent, J., *The need for selenite and molybdate in the formation of formic dehydrogenase by members of the Coli-aerogenes group of bacteria.* Biochem J, 1954. **57**(1): p. 10-16.
66. Schwarz, K. and C.M. Foltz, *Selenium as an Integral Part of Factor-3 against Dietary Necrotic Liver Degeneration.* Journal of the American Chemical Society, 1957. **79**(12): p. 3292-3293.
67. Diplock, A.T., *Indexes of selenium status in human populations.* Am J Clin Nutr, 1993. **57**(2 Suppl): p. 256S-258S.
68. Fordyce, F.M., et al., *Soil, grain and water chemistry in relation to human selenium-responsive diseases in Enshi District, China.* App Geochem, 2000. **15**(1): p. 117-132.
69. Johnson, C.C., et al., *Selenium distribution in the local environment of selected villages of the Keshan Disease belt, Zhangjiakou District, Hebei Province, People's Republic of China.* Appl Geochem, 2000. **15**(3): p. 385-401.
70. Adams, M.L., et al., *Evidence of low selenium concentrations in UK bread-making wheat grain.* Journal of the Science of Food and Agriculture, 2002. **82**(10): p. 1160-1165.
71. Dumont, E., F. Vanhaecke, and R. Cornelis, *Selenium speciation from food source to metabolites: a critical review.* Analytical and Bioanalytical Chemistry, 2006. **385**(7): p. 1304-1323.
72. Geering, H.R., et al., *Solubility and redox criteria for the possible forms of selenium in soils.* Soil Science Society of America Journal, 1968. **32**(1): p. 35-40.
73. Rayman, M.P., *Food-chain selenium and human health: emphasis on intake.* Br J Nutr, 2008. **100**(2): p. 254-268.
74. Kabata-Pendias, A., *Trace elements in plants*, in *Trace elements in soils and plants, fourth edition*. 2011, CRC Press. p. 93-121.
75. Mayland, H.F. *Selenium in Soils and Plants*. in *Selenium Responsive Diseases in Food Animals. Western States Veterinary Conference*. 1985. Las Vegas, Nevada: Veterinary Learning Systems Co., Inc., Princeton Junction, NJ.
76. Rayman, M.P., *The use of high-selenium yeast to raise selenium status: how does it measure up?* Br J Nutr, 2004. **92**(4): p. 557-573.
77. Combs, G.F., Jr., *Selenium in foods.* Adv Food Res, 1988. **32**: p. 85-113.
78. Hakkarainen, J. *Bioavailability of selenium*. in *NJF symposium: Problems on selenium in animal nutrition*. 1993. Ås, Norway: Norwegian Agricultural Advisory Service, Norway.
79. Butler, J.A., M.A. Beilstein, and P.D. Whanger, *Influence of dietary methionine on the metabolism of selenomethionine in rats.* J Nutr, 1989. **119**(7): p. 1001-1009.
80. Fairweather-Tait, S.J., *Bioavailability of selenium.* Eur J Clin Nutr, 1997. **51 Suppl 1**: p. S20-S23.

81. Underwood, E.J., *Selenium*, in *Trace Elements in Human and Animal Nutrition*. 1977, Academic Press, Inc.: New York. p. 302-340.
82. Vendeland, S.C., J.A. Butler, and P.D. Whanger, *Intestinal absorption of selenite, selenate, and selenomethionine in the rat.* J. Nutr. Biochem., 1992. **3**(7): p. 359-365.
83. Templeton, D.M., et al., *Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000).* Pure and Applied Chemistry, 2000. **72**(8): p. 1453-1470.
84. Reilly, C., *Selenium in food and health*. 1996, London ; New York: Blackie Academic & Professional. xvi, 338 p.
85. Clausen, J. and S.A. Nielsen, *Comparison of whole blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenate, selenite,l-selenomethionine, and high selenium yeast.* Biol Trace Elem Res, 1988. **15**(1): p. 125-138.
86. Schrauzer, G.N. and D.A. White, *Selenium in human nutrition: Dietary intakes and effects of supplementation.* Bioinorg Chem, 1978. **8**(4): p. 303-318.
87. Alexander, J., et al., *Selenium in clinical medicine and medical biochemistry.* Klinisk biokemi i Norden, 2019. **31**(3): p. 12-19.
88. Deniziak, M., et al., *Loss of selenoprotein N function causes disruption of muscle architecture in the zebrafish embryo.* Exp Cell Res, 2007. **313**(1): p. 156-167.
89. Stadtman, T.C., *Selenocysteine.* Annu Rev Biochem, 1996. **65**(1): p. 83-100.
90. Kryukov, G.V., et al., *Characterization of Mammalian Selenoproteomes.* Science, 2003. **300**(5624): p. 1439-1443.
91. Lee, S.-R., et al., *Mammalian thioredoxin reductase: Oxidation of the C-terminal cysteine/selenocysteine active site forms a thioselenide, and replacement of selenium with sulfur markedly reduces catalytic activity.* Proc Natl Acad Sci U S A, 2000. **97**(6): p. 2521-2526.
92. Combs, G.F., Jr., *Selenium in global food systems.* Br J Nutr, 2001. **85**(5): p. 517-547.
93. Lu, J., C. Berndt, and A. Holmgren, *Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase.* Biochim Biophys Acta, 2009. **1790**(11): p. 1513-9.
94. Jönsson-Videsäter, K., et al., *Selenite-induced apoptosis in doxorubicin-resistant cells and effects on the thioredoxin system.* Biochemical Pharmacology, 2004. **67**(3): p. 513-522.
95. Tamura, T. and T.C. Stadtman, *A new selenoprotein from human lung adenocarcinoma cells: purification, properties, and thioredoxin reductase activity.* Proc Natl Acad Sci USA, 1996. **93**(3): p. 1006-1011.
96. Haratake, M., et al., *Selenium binding to human hemoglobin via selenotrisulfide.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2005. **1723**(1–3): p. 215-220.

97. Zhang, Y., et al., *Role of selenoproteins in redox regulation of signaling and the antioxidant system: a review.* Antioxidants, 2020. **9**(5): p. 383-399.
98. Behne, D., et al., *Evidence for specific selenium target tissues and new biologically important selenoproteins.* Biochimica et Biophysica Acta (BBA) - General Subjects, 1988. **966**(1): p. 12-21.
99. Schomburg, L. and U. Schweizer, *Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium.* Biochim Biophys Acta Gen Subj, 2009. **1790**(11): p. 1453-1462.
100. Olson, G.E., et al., *Apolipoprotein E receptor-2 (ApoER2) mediates selenium uptake from selenoprotein P by the mouse testis.* J. Biol. Chem., 2007. **282**(16): p. 12290-12297.
101. Olson, G.E., et al., *Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells.* J Biol Chem, 2008. **283**(11): p. 6854-6860.
102. Kurokawa, S., et al., *Long isoform mouse selenoprotein P (Sepp1) supplies rat myoblast L8 cells with selenium via endocytosis mediated by heparin binding properties and apolipoprotein E receptor-2 (ApoER2).* The Journal of biological chemistry, 2012. **287**(34): p. 28717-28726.
103. Hill, K.E., et al., *Production of selenoprotein P (Sepp1) by hepatocytes is central to selenium homeostasis.* J Biol Chem, 2012. **287**(48): p. 40414-24.
104. McCann, J.C. and B.N. Ames, *Adaptive dysfunction of selenoproteins from the perspective of the triage theory: why modest selenium deficiency may increase risk of diseases of aging.* FASEB J, 2011. **25**(6): p. 1793-1814.
105. Schomburg, L., et al., *Synthesis and metabolism of thyroid hormones is preferentially maintained in selenium-deficient transgenic mice.* Endocrinology, 2006. **147**(3): p. 1306-1313.
106. Schomburg, L. and J. Köhrle, *On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health.* Mol Nutr Food Res, 2008. **52**(11): p. 1235-1246.
107. Lubos, E., J. Loscalzo, and D.E. Handy, *Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities.* Antioxid Redox Signal, 2011. **15**(7): p. 1957-1597.
108. Florian, S., et al., *Loss of GPx2 increases apoptosis, mitosis, and GPx1 expression in the intestine of mice.* Free Radic Biol Med, 2010. **49**(11): p. 1694-1702.
109. Combs, J., Gerald F., *Biomarkers of selenium status.* Nutrients, 2015. **7**(4): p. 2209-2236.
110. Ursini, F., et al., *Dual function of the selenoprotein PHGPx during sperm maturation.* Science, 1999. **285**(5432): p. 1393-1396.
111. Foresta, C., et al., *Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase.* Biol Reprod, 2002. **67**(3): p. 967-971.

112. Maiorino, M., et al., *Genetic variations of gpx-4 and male infertility in humans.* Biol Reprod, 2003. **68**(4): p. 1134-1141.
113. Yant, L.J., et al., *The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults.* Free Radic Biol Med, 2003. **34**(4): p. 496-502.
114. Ursini, F. and A. Bindoli, *The role of selenium peroxidases in the protection against oxidative damage of membranes.* Chem Phys Lipids, 1987. **44**(2): p. 255-276.
115. Cox, A.G., et al., *Selenoprotein H is an essential regulator of redox homeostasis that cooperates with p53 in development and tumorigenesis.* Proc Natl Acad Sci U S A, 2016. **113**(38): p. E5562-E5571.
116. Wu, R.T.Y., et al., *Selenoprotein H suppresses cellular senescence through genome maintenance and redox regulation.* J Biol Chem, 2014. **289**(49): p. 34378-34388.
117. Novoselov, S.V., et al., *Selenoprotein H is a nucleolar thioredoxin-like protein with a unique expression pattern.* J Biol Chem, 2007. **282**(16): p. 11960-11968.
118. Korotkov, K.V., et al., *Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element.* Mol Cell Biol, 2002. **22**(5): p. 1402-1411.
119. Hwang, D.Y., et al., *Overexpression of human selenoprotein M differentially regulates the concentrations of antioxidants and H2O2, the activity of antioxidant enzymes, and the composition of white blood cells in a transgenic rat.* Int J Mol Med, 2008. **21**(2): p. 169-179.
120. Sengupta, A., et al., *Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells.* Biochem Cell Biol, 2009. **87**(6): p. 953-961.
121. Dikiy, A., et al., *SelT, SelW, SelH, and Rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family.* Biochemistry, 2007. **46**(23): p. 6871-6882.
122. Chung, Y.W., et al., *Antioxidative role of selenoprotein W in oxidant-induced mouse embryonic neuronal cell death.* Mol Cells, 2009. **27**(5): p. 609-613.
123. Jeong, D.-w., et al., *Selenoprotein W is a glutathione-dependent antioxidant in vivo.* FEBS Lett., 2002. **517**(1-3): p. 225-228.
124. Vendeland, S.C., et al., *Purification and properties of selenoprotein W from rat muscle.* J Biol Chem, 1993. **268**(23): p. 17103-17107.
125. Lee, S.R., et al., *Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver.* J Biol Chem, 1999. **274**(8): p. 4722-4734.
126. Sun, Q.A., et al., *Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases.* J Biol Chem, 1999. **274**(35): p. 24522-24530.

127. Lu, C., et al., *Identification and characterization of selenoprotein K: An antioxidant in cardiomyocytes.* FEBS Lett, 2006. **580**(22): p. 5189-5197.
128. Du, S., et al., *SelK is a novel ER stress-regulated protein and protects HepG2 cells from ER stress agent-induced apoptosis.* Arch Biochem Biophys, 2010. **502**(2): p. 137-143.
129. Lee, J.H., et al., *Selenoprotein S-dependent selenoprotein K binding to p97(VCP) protein is essential for endoplasmic reticulum-associated degradation.* J Biol Chem, 2015. **290**(50): p. 29941-29952.
130. Han, S.-J., et al., *Characterization of mammalian selenoprotein O: a redox-active mitochondrial protein.* PLOS One, 2014. **9**(4): p. e95518 (7 p.).
131. Saito, Y., et al., *Selenoprotein P in human plasma as an extracellular phospholipid hydroperoxide glutathione peroxidase: Isolation and enzymatic characterization of human selenoprotein P.* J Biol Chem, 1999. **274**(5): p. 2866-2871.
132. Du, S., H. Liu, and K. Huang, *Influence of SelS gene silence on beta-Mercaptoethanol-mediated endoplasmic reticulum stress and cell apoptosis in HepG2 cells.* Biochim Biophys Acta, 2010. **1800**(5): p. 511-517.
133. Xu, X.M., et al., *New developments in selenium biochemistry: selenocysteine biosynthesis in eukaryotes and archaea.* Biol Trace Elem Res, 2007. **119**(3): p. 234-41.
134. Xu, X.-M., et al., *Selenophosphate synthetase 2 is essential for selenoprotein biosynthesis.* Biochem J, 2007. **404**(Pt 1): p. 115-120.
135. Kipp, A., et al., *Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon.* Mol Nutr Food Res, 2009. **53**(12): p. 1561-1572.
136. Kipp, A.P., et al., *Selenoprotein W as biomarker for the efficacy of selenium compounds to act as source for selenoprotein biosynthesis.* Methods Enzymol, 2013. **527**: p. 87-112.
137. Karunasinghe, N., et al., *DNA stability and serum selenium levels in a high-risk group for prostate cancer.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(3): p. 391-397.
138. Arthur, J.R., R.C. McKenzie, and G.J. Beckett, *Selenium in the immune system.* J Nutr, 2003. **133**(5): p. 1457-1459.
139. Boosalis, M.G., *The role of selenium in chronic disease.* Nutr Clin Pract, 2008. **23**(2): p. 152-160.
140. Rayman, M.P., *The importance of selenium to human health.* Lancet, 2000. **356**(9225): p. 233-241.
141. Burbano, X., et al., *Impact of a selenium chemoprevention clinical trial on hospital admissions of HIV-infected participants.* HIV Clin Trials, 2002. **3**(6): p. 483-491.
142. Kamwesiga, J., et al., *Effect of selenium supplementation on CD4+ T-cell recovery, viral suppression and morbidity of HIV-infected patients in*

Rwanda: a randomized controlled trial. AIDS, 2015. **29**(9): p. 1045-1052.
143. Verma, S., et al., *Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses.* J Immunol, 2011. **186**(4): p. 2127-2137.
144. Rock, C. and P.J. Moos, *Selenoprotein P protects cells from lipid hydroperoxides generated by 15-LOX-1.* Prostaglandins Leukot Essent Fatty Acids, 2010. **83**(4-6): p. 203-210.
145. Humann-Ziehank, E., et al., *Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae.* Metallomics, 2014. **6**(10): p. 1869-1879.
146. Angstwurm, M.W., et al., *Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome.* Crit Care Med, 1999. **27**(9): p. 1807-1813.
147. Maehira, F., et al., *Alterations of serum selenium concentrations in the acute phase of pathological conditions.* Clin Chim Acta, 2002. **316**(1–2): p. 137-146.
148. Sun, L.-H., et al., *Response of selenium and selenogenome in immune tissues to LPS-induced inflammatory reactions in pigs.* Biological Trace Element Research, 2017. **177**(1): p. 90-96.
149. Manzanares, W., et al., *Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill?* Intensive Care Med, 2009. **35**(5): p. 882-889.
150. Hollenbach, B., et al., *New assay for the measurement of selenoprotein P as a sepsis biomarker from serum.* Journal of Trace Elements in Medicine and Biology, 2008. **22**(1): p. 24-32.
151. Becker, N.-P., et al., *Hypoxia reduces and redirects selenoprotein biosynthesis.* Metallomics, 2014. **6**(5): p. 1079-1086.
152. Stoedter, M., et al., *Selenium controls the sex-specific immune response and selenoprotein expression during the acute-phase response in mice.* Biochemical Journal, 2010. **429**(1): p. 43-51.
153. Schweizer, U., et al., *Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply.* Biochem J, 2005. **386**(2): p. 221-226.
154. Olson, G.E., et al., *Megalin Mediates Selenoprotein P Uptake by Kidney Proximal Tubule Epithelial Cells.* Journal of Biological Chemistry, 2008. **283**(11): p. 6854-6860.
155. Sammalkorpi, K., et al., *Serum selenium in acute infections.* Infection, 1988. **16**(4): p. 222-224.
156. Vonnahme, K.A., et al., *Supranutritional selenium increases mammary gland vascularity in postpartum ewe lambs.* J. Dairy Sci., 2011. **94**(6): p. 2850-2858.
157. Hall, J.A., et al., *Effect of supranutritional organic selenium supplementation on postpartum blood micronutrients, antioxidants,*

metabolites, and inflammation biomarkers in selenium-replete dairy cows. Biol Trace Elem Res, 2014. **161**(3): p. 272-287.
158. Hall, J.A., et al., *Effect of supranutritional maternal and colostral selenium supplementation on passive absorption of immunoglobulin G in selenium-replete dairy calves.* J. Dairy Sci., 2014. **97**(7): p. 4379-4391.
159. Taylor, J.B., *Time-dependent influence of supranutritional organically bound selenium on selenium accumulation in growing wether lambs.* J Anim Sci, 2005. **83**(5): p. 1186-93.
160. Ueno, H., et al., *Effects of selenium status and supplementary seleno-chemical sources on mouse T-cell mitogenesis.* J Trace Elem Med Biol, 2008. **22**(1): p. 9-16.
161. Borella, P., A. Bargellini, and C.I. Medici, *Chemical form of selenium greatly affects metal uptake and responses by cultured human lymphocytes.* Biol Trace Elem Res, 1996. **51**(1): p. 43-54.
162. Rayman, M.P., *Food-chain selenium and human health: emphasis on intake.* British Journal of Nutrition, 2008. **100**(2): p. 254-268.
163. Thomson, C.D., *Assessment of requirements for selenium and adequacy of selenium status: a review.* Eur J Clin Nutr, 2004. **58**(3): p. 391-402.
164. Groce, A.W., et al., *Levels of selenium and other essential minerals in forages, bovine blood and serum relative to soil type.* Vet Clin Nutr, 1995. **2**: p. 146-152.
165. Frøslie, A., *Selenium deficiency diseases and their prevention in animal husbandry in Norway*, in *Human and animal health in relation to circulation processes of selenium and cadmium*, J. Låg, Editor. 1991, The Norwegian Academy of Science and Letters: Oslo. p. 213-221.
166. Flores-Mateo, G., et al., *Selenium and coronary heart disease: a meta-analysis.* Am J Clin Nutr, 2006. **84**(4): p. 762-773.
167. Lee, E.H., et al., *Effects of selenium supplements on cancer prevention: meta-analysis of randomized controlled trials.* Nutr Cancer, 2011. **63**(8): p. 1185-1195.
168. Clark, L.C., et al., *Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial.* J Am Med Assoc, 1996. **276**(24): p. 1957-1963.
169. Beck, M.A., et al., *Selenium deficiency increases the pathology of an influenza virus infection.* FASEB J, 2001. **15**(8): p. 1481-1483.
170. Parnham, M.J., J. Winkelmann, and S. Leyck, *Macrophage, lymphocyte and chronic inflammatory responses in selenium deficient rodents. Association with decreased glutathione peroxidase activity.* Int J Immunopharmacol, 1983. **5**(5): p. 455-461.
171. Boyne, R. and J.R. Arthur, *Alterations of neutrophil function in selenium-deficient cattle.* J Comp Pathol, 1979. **89**(1): p. 151-158.
172. Rayman, M.P., *Selenium and human health.* Lancet, 2012. **379**(9822): p. 1256-1268.

173. Ceko, M.J., et al., *X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function.* Metallomics, 2015. **7**(1): p. 71-82.
174. Imai, H., et al., *Failure of the expression of phospholipid hydroperoxide glutathione peroxidase in the spermatozoa of human infertile males.* Biol Reprod, 2001. **64**(2): p. 674-683.
175. Hill, K.E., et al., *Thioredoxin reductase activity is decreased by selenium deficiency.* Biochem Biophys Res Commun, 1997. **234**(2): p. 293-295.
176. Combs Jr, G.F. and S.B. Combs, *Selenium and cancer*, in *The role of selenium in nutrition*. 1986, Academic Press. p. 413-461.
177. Pedrero, Z. and Y. Madrid, *Novel approaches for selenium speciation in foodstuffs and biological specimens: a review.* Anal Chim Acta, 2009. **634**(2): p. 135-152.
178. Lescure, A., et al., *Selenoprotein function and muscle disease.* Biochim Biophys Acta, 2009. **1790**(11): p. 1569-1574.
179. Kontush, A., et al., *Antioxidant and prooxidant activity of alpha-tocopherol in human plasma and low density lipoprotein.* J Lipid Res, 1996. **37**(7): p. 1436-1448.
180. Surai, P.F., *Vitamin E in avian reproduction.* Poult Avian Biol Rev, 1999. **10**(1): p. 1-60.
181. Trapp, A.L., et al., *Vitamin E-selenium deficiency in swine: differential diagnosis and nature of field problem.* J Am Vet Med Assoc, 1970. **157**: p. 289-300.
182. Wuryastuti, H., et al., *Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum, and milk leukocytes of sows.* J Anim Sci, 1993. **71**(9): p. 2464-2472.
183. Moir, D.C. and H.G. Masters, *Hepatosis Dietetica, Nutritional Myopathy, Mulberry Heart Disease and associated hepatic selenium levels in pigs.* Aust Vet J, 1979. **55**(8): p. 360-364.
184. Grant, C.A., *Morphological and aetiological studies of dietetic microangiopathy in pigs (" mulberry heart ").* Acta Veterinaria Scandinavica, 1961. **2**(Suppl. 3): p. 107 pp.
185. Frøslie, A., J.T. Karlsen, and J. Rygge, *Selenium in animal nutrition in Norway.* Acta Agriculturae Scandinavica, 1980. **30**(1): p. 17-25.
186. Wu, X. and J. Låg, *Selenium in Norwegian farmland soils.* Acta Agric Scand, 1988. **38**(3): p. 271-276.
187. Øvernes, G. and A. Frøslie. *Geographical distribution of iodine and selenium deficiency in animals*. in *Chemical climatology and geomedical problems.* 1992. Oslo, Norway: Universitetsforlaget AS, Norway.
188. Sivertsen, T., et al., *Geographical and botanical variation in concentrations of selenium, cobalt, iodine, zinc and other essential elements in sheep pasture plants in Norway.* Acta Agric Scand A — Anim Sci, 2014. **64**(3): p. 188-197.

189. Alonso-Alvarez, C., et al., *Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches.* Funct Ecol, 2007. **21**(5): p. 873-879.
190. Brown-Borg, H.M. and S.G. Rakoczy, *Growth hormone administration to long-living dwarf mice alters multiple components of the antioxidative defense system.* Mech Ageing Dev, 2003. **124**(10–12): p. 1013-1024.
191. Inness, C.L. and N.B. Metcalfe, *The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish.* Proc Biol Sci, 2008. **275**(1644): p. 1703-1708.
192. Pallarés, F.J., et al., *Vitamin E and selenium concentrations in livers of pigs diagnosed with mulberry heart disease.* J Vet Diagn Invest, 2002. **14**(5): p. 412-414.
193. Norwegian Meat and Poultry Research Center, *Helsetjenesten for svin - Årsrapport 2011 [Pig Health Services - Annual report 2011]*. 2012: http://www.animalia.no/upload/FIler%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport2011.pdf.
194. Blanckenhorn, W.U., *The evolution of body size: What keeps organisms small?* Q Rev Biol, 2000. **75**(4): p. 385-407.
195. Arendt, J.D., *Adaptive intrinsic growth rates: An integration across taxa.* Q Rev Biol, 1997. **72**(2): p. 149-177.
196. Shen, H., et al., *Vitamin E and selenium levels are within normal range in pigs diagnosed with Mulberry Heart Disease and evidence for viral involvement in the syndrome is lacking.* Transbound Emerg Dis, 2011. **58**(6): p. 483-491.
197. Lauridsen, C. and S.K. Jensen, *Influence of supplementation of all-rac-α-tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and immune responses of piglets.* J Anim Sci, 2005. **83**(6): p. 1274-1286.
198. Dubey, A., et al., *Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse.* Arch Biochem Biophys, 1996. **333**(1): p. 189-197.
199. Pauliny, A., et al., *Rapid growth accelerates telomere attrition in a transgenic fish.* BMC Evol Biol, 2015. **15**(1): p. 159-168.
200. Cooper, B.J. and B.A. Valentine, *Muscle and Tendon*, in *Jubb, Kennedy & Palmer's Pathology of Domestic Animals* 2015: Philadelphia, USA. p. 164-249.
201. Nielsen, T.K., et al., *Mulberry Heart Disease in young pigs without vitamin E and selenium deficiency.* Vet Rec, 1989. **124**(20): p. 535-537.
202. Mahan, D.C. and J.C. Peters, *Long-term effects of dietary organic and inorganic selenium sources and levels on reproducing sows and their progeny.* J Anim Sci, 2004. **82**(5): p. 1343-1358.
203. Combs, G.F., Jr., *Impact of selenium and cancer-prevention findings on the nutrition-health paradigm.* Nutr Cancer, 2001. **40**(1): p. 6-11.

204. EFSA, *Scientific opinion on safety and efficacy of Sel-Plex® (organic form of selenium produced by Saccharomyces cerevisiae CNCM I-3060) for all species.*, in *EFSA Journal*. 2011. p. 2110 [52 pp.].
205. Penglase, S., K. Hamre, and S. Ellingsen, *The selenium content of SEPP1 versus selenium requirements in vertebrates.* PeerJ, 2015. **3**: p. e1244.
206. FAO/WHO, *Vitamin and mineral requirements in human nutrition*. 2nd ed. 2004, Geneva, Rome: World Health Organization (WHO); Food and Agriculture Organization of the United Nations (FAO). xix, 341 p.
207. Kipp, A.P., et al., *Revised reference values for selenium intake.* J Trace Elem Med Biol, 2015. **32**: p. 195-199.
208. Kieliszek, M. and S. Błażejak, *Selenium: Significance, and outlook for supplementation.* Nutrition, 2013. **29**(5): p. 713-718.
209. Tinggi, U., *Essentiality and toxicity of selenium and its status in Australia: a review.* Toxicol Lett, 2003. **137**(1–2): p. 103-110.
210. Rayman, M.P., *Dietary selenium: time to act.* BMJ, 1997. **314**(7078): p. 387-388.
211. Ellingsen, D.G., et al., *The time-trend and the relation between smoking and circulating selenium concentrations in Norway.* J Trace Elem Med Biol, 2009. **23**(2): p. 107-15.
212. MacPherson, A., et al. *Loss of Canadian wheat imports lowers selenium intake and status of the Scottish population*. in *Trace Elements in Man and Animals 9*. 1997. Ottawa: NRC Research Press.
213. UniProtKB. *UniProtKB - A1E951 (A1E951_PIG)*. [cited 2017 18.04.2017]; Available from: http://www.uniprot.org/uniprot/A1E951.
214. National Research Council, *Nutrient requirements of swine: eleventh revised edition*. 2012, Washington, DC: The National Academies Press. 400.
215. Tybirk, P., et al., *Normer for næringsstoffer. [Standards on nutrients]*. 2015, VIDENCENTER FOR SVINEPRODUKTION.
216. Eur Comm, *COUNCIL DIRECTIVE of 23 November 1970 concerning additives in feeding-stuffs (70/524/EEC).* OJEC, 1970(L 270/1).
217. Schrauzer, G.N., *Nutritional selenium supplements: product types, quality, and safety.* J Am Coll Nutr, 2001. **20**(1): p. 1-4.
218. Frøslie, A., K. Moksnes, and G. Øvernes, *The effect of selenium supplementation of animal feeds in Norway.* Acta Agric Scand, 1985. **35**(2): p. 139-144.
219. Nafstad, I. and S. Tollersrud, *The vitamin E-deficiency syndrome in pigs. I. Pathological changes.* Acta Vet Scand, 1970. **11**(3): p. 452-480.
220. van Kempen, T.A., et al., *Vitamin E plasma kinetics in swine show low bioavailability and short half-life of alpha-tocopheryl acetate.* J Anim Sci, 2016. **94**(10): p. 4188-4195.
221. Jensen, S.K. and C. Lauridsen, *α-Tocopherol stereoisomers*, in *Vitamins and Hormones*. 2007, Academic Press. p. 281-308.

222. Alfthan, G., et al., *Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate.* Am J Clin Nutr, 1991. **53**(1): p. 120-125.
223. Levander, O.A., et al., *Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters.* Am J Clin Nutr, 1983. **37**(6): p. 887-897.
224. Pérez-Corona, M.T., et al., *Selenium biotransformation by Saccharomyces cerevisiae and Saccharomyces bayanus during white wine manufacture: Laboratory-scale experiments.* Food Chem, 2011. **124**(3): p. 1050-1055.
225. Beilstein, M.A. and P.D. Whanger, *Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine.* J Nutr, 1986: p. 1711-1719.
226. EFSA, *Opinion of the panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product Sel-Plex 2000 as a feed additive according to Regulation (EC) No 1831/2003.* EFSA J, 2006. **4**(5): p. 348-n/a.
227. Uden, P.C., et al., *Element selective characterization of stability and reactivity of selenium species in selenized yeast.* J. Anal. At. Spectrom., 2004. **19**(1): p. 65-73.
228. Lamont, H.G., D. Luke, and W.A.M. Gordon, *Some pig diseases.* Veterinary Record, 1950. **62**: p. 737-743.
229. Sharp, B.A., L.G. Young, and A.A. Van Dreumel, *Dietary induction of mulberry heart disease and hepatosis dietetica in pigs. I. Nutritional aspects.* Canadian journal of comparative medicine : Revue canadienne de medecine comparee, 1972. **36**(4): p. 371-376.
230. Mattilsynet [Norwegian Food Safety Authority], *FOR-2005-04-12-319 Forskrift om tilsetningsstoffer til bruk i fôrvarer. [Regulation on feed additives]*, in *nr. 1750/2006*. 2007: https://lovdata.no/dokument/SF/forskrift/2005-04-12-319.
231. Mattilsynet [Norwegian Food Safety Authority], *FOR-2014-01-20-50 Forskrift om endring i forskrift om tilsetningsstoffer til bruk i fôrvarer. [Regulation on the amendment of the regulation on feed additives]*, in *20 januar 2014 nr. 50*. 2014: I 2014 hefte 1 s 127.
232. Ponce de León, C.A., et al., *Selenium incorporation into Saccharomyces cerevisiae cells: a study of different incorporation methods.* J Appl Microbiol, 2002. **92**(4): p. 602-610.
233. Larsen, E.H., et al., *Selenium speciation and isotope composition in 77Se-enriched yeast using gradient elution HPLC separation and ICP-dynamic reaction cell-MS.* J Anal At Spectrom, 2003. **18**(4): p. 310-316.
234. Sloth, J.J., et al., *Determination of total selenium and 77Se in isotopically enriched human samples by ICP-dynamic reaction cell-MS.* J Anal At Spectrom, 2003. **18**(4): p. 317-322.

235. Fox, T.E., et al., *Bioavailability of selenium from fish, yeast and selenate: a comparative study in humans using stable isotopes.* Eur J Clin Nutr, 2004. **58**(2): p. 343-349.
236. EFSA, *Scientific opinion on the safety and efficacy of L-selenomethionine as feed additive for all animal species.* EFSA J, 2013. **11**(5): p. 3219-3237.
237. Zhan, X., et al., *Selenomethionine: an effective selenium source for sow to improve Se distribution, antioxidant status, and growth performance of pig offspring.* Biol Trace Elem Res, 2011. **142**(3): p. 481-491.
238. Behne, D. and A. Kyriakopoulos, *Mammalian selenium-containing proteins.* Annu Rev Nutr, 2001. **21**: p. 453-473.
239. Windisch, W., S. Gabler, and M. Kirchgessner, *Effect of selenite, seleno cysteine and seleno methionine on the selenium metabolism of ^{75}Se labeled rats.* J Anim Physiol Anim Nutr, 1998. **78**(1-5): p. 67-74.
240. Yang, G., et al., *Studies of safe maximal daily dietary Se-intake in a seleniferous area in China. Part II: Relation between Se-intake and the manifestation of clinical signs and certain biochemical alterations in blood and urine.* J Trace Elem Electrolytes Health Dis, 1989. **3**(3): p. 123-130.
241. Sunde, R.A., *Selenium.*, in *Handbook of nutritionally essential mineral elements.*, B.L. O'Dell and R.A. Sunde, Editors. 1997, Marcel Dekker, Inc.: New York, USA. p. 493-556.
242. Hawkes, W.C., E.C. Wilhelmsen, and A.L. Tappel, *Abundance and tissue distribution of selenocysteine-containing proteins in the rat.* J Inorg Biochem, 1985. **23**(2): p. 77-92.
243. Schrauzer, G.N., *Selenomethionine: a review of its nutritional significance, metabolism and toxicity.* J Nutr, 2000. **130**(7): p. 1653-1656.
244. Sunde, R.A., G.E. Gutzke, and W.G. Hoekstra, *Effect of dietary methionine on the biopotency of selenite and selenomethionine in the rat.* J Nutr, 1981. **111**(1): p. 76-88.
245. Behne, D. and W. Wolters, *Distribution of selenium and glutathione peroxidase in the rat.* J Nutr, 1983. **113**(2): p. 456-61.
246. Burk, R.F., et al., *Influence of dietary and injected selenium on whole-body retention, route of excretion, and tissue retention of 75SeO3 2- in the rat.* J Nutr, 1972. **102**(8): p. 1049-55.
247. Fairweather-Tait, S.J., R. Collings, and R. Hurst, *Selenium bioavailability: current knowledge and future research requirements.* Am J Clin Nutr, 2010. **91**(5): p. 1484S-1491S.
248. Kieliszek, M. and S. Błażejak, *Current knowledge on the importance of selenium in food for living organisms: A review.* Molecules, 2016. **21**(5): p. 609-624.
249. Gissel-Nielsen, G., *Effects of selenium supplementation of field crops.*, in *Environmental chemistry of selenium*, W.T. Frankenberger and R.A. Engberg, Editors. 1998, Marcel Dekker, Inc.: New York. p. 99-112.

250. Thomson, C.D. and M.F. Robinson, *The changing selenium status of New Zealand residents.* Eur J Clin Nutr, 1996. **50**(2): p. 107-114.
251. Alfthan, G., et al., *Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population.* J Trace Elem Med Biol, 2015. **31**: p. 142-147.
252. Vannoort, R., P. Cressey, and K. Silvers, *1997/98 New Zealand total diet survey. Part 2 : ELEMENTS - Selected contaminants & nutrients*. 2000, Ministry of Health contract for scientific services: Ministry of Health's web site.
253. Holben, D.H. and A.M. Smith, *The Diverse Role of Selenium within Selenoproteins.* J Am Diet Assoc, 1999. **99**(7): p. 836-843.
254. Maseko, T., et al., *Chemical characterisation and speciation of organic selenium in cultivated selenium-enriched Agaricus bisporus.* Food Chem, 2013. **141**(4): p. 3681-3687.
255. Waegeneers, N., et al., *Predicted dietary intake of selenium by the general adult population in Belgium.* Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2013. **30**(2): p. 278-285.
256. Choi, Y., et al., *Selenium content in representative Korean foods.* Journal of Food Composition and Analysis, 2009. **22**(2): p. 117-122.
257. Pehrson, B., *Organic selenium for supplementation of farm animal diets: it's influence on the selenium status of the animals and on the dietary selenium intake of man. in: Re-defining mineral nutrition.* 2005: p. 253-267.
258. Cole, D.J.A., *Selenium, the pig and the human diet.*, in *Concepts in Pig Science*, T.P. Lyons and D.J.A. Cole, Editors. 2000, Nottingham University Press: Nottingham, UK. p. 149–158.
259. Rigotti, A., *Absorption, transport, and tissue delivery of vitamin E.* Mol Aspects Med, 2007. **28**(5-6): p. 423-436.
260. Burton, G.W., A. Joyce, and K.U. Ingold, *First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma.* Lancet, 1982. **2**(8293): p. 327.
261. Burton, G.W., A. Joyce, and K.U. Ingold, *Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes?* Arch Biochem Biophys, 1983. **221**(1): p. 281-290.
262. Ingold, K.U., et al., *Vitamin E remains the major lipid-soluble, chain-breaking antioxidant in human plasma even in individuals suffering severe vitamin E deficiency.* Arch Biochem Biophys, 1987. **259**(1): p. 224-225.
263. Brown, K.M., P.C. Morrice, and G.G. Duthie, *Erythrocyte membrane fatty acid composition of smokers and non-smokers: effects of vitamin E supplementation.* Eur J Clin Nutr, 1998. **52**(2): p. 145-150.
264. Wang, F., et al., *Vitamin E inhibits hemolysis induced by hemin as a membrane stabilizer.* Biochem Pharmacol, 2006. **71**(6): p. 799-805.

265. Gradinski-Vrbanac, B., et al., *In vitro susceptibility of duck, chicken, and pig erythrocyte lipids to peroxidation.* Vet Med-Czech, 2002. **47**(10-11): p. 303-308.
266. Holvoet, P. and D. Collen, *Oxidized lipoproteins in atherosclerosis and thrombosis.* FASEB J, 1994. **8**(15): p. 1279-1284.
267. Moreira, I. and D.C. Mahan, *Effect of dietary levels of vitamin E (all-rac-tocopheryl acetate) with or without added fat on weanling pig performance and tissue alpha-tocopherol concentration.* J Anim Sci, 2002. **80**(3): p. 663-669.
268. Jensen, M., A. Lindholm, and J. Hakkarainen, *The vitamin E distribution in serum, liver, adipose and muscle tissues in the pig during depletion and repletion.* Acta Vet Scand, 1990. **31**(2): p. 129-136.
269. Jensen, M., et al., *Vitamin E requirement of growing swine.* J Anim Sci, 1988. **66**(12): p. 3101-3111.
270. Flory, S., M. Birringer, and J. Frank, *Bioavailability and metabolism of vitamin E*, in *Vitamin E in human health*, P. Weber, et al., Editors. 2019, Springer International Publishing: Cham, DE. p. 31-41.
271. Russell, W.M.S. and R.L. Burch, *The sources, incidence, and removal of inhumanity.*, in *The principles of humane experimental technique.* 1959, Methuen & Co.: Wheathampstead, UK: Universities Federation for Animal Welfare.
272. Merlot, E., A.M. Mounier, and A. Prunier, *Endocrine response of gilts to various common stressors: a comparison of indicators and methods of analysis.* Physiol Behav, 2011. **102**(3-4): p. 259-65.
273. Marchant-Forde, J. and R.M. Marchant-Forde, *Welfare of pigs during transport and slaughter*. 2009. p. 301-330.
274. Campbell, J.M., J.D. Crenshaw, and J. Polo, *The biological stress of early weaned piglets.* J Anim Sci Biotechnol, 2013. **4**(1): p. 19.
275. Brown, S.N., et al., *The response of pigs to being loaded or unloaded onto commercial animal transporters using three systems.* Vet J, 2005. **170**(1): p. 91-100.
276. Raetz, C.R.H. and C. Whitfield, *Lipopolysaccharide endotoxins.* Annu Rev Biochem, 2002. **71**(1): p. 635-700.
277. Redl, H., et al., *Clinical detection of LPS and animal models of endotoxemia.* Immunobiology, 1993. **187**(3): p. 330-345.
278. Weber, T.E. and B.J. Kerr, *Effect of sodium butyrate on growth performance and response to lipopolysaccharide in weanling pigs.* J Anim Sci, 2008. **86**(2): p. 442-450.
279. Carroll, J.A., et al., *Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: II. Effects on the hypothalamic-pituitary-adrenal axis of weaned pigs.* J Anim Sci, 2002. **80**(2): p. 502-509.
280. Touchette, K.J., et al., *Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs.* J Anim Sci, 2002. **80**(2): p. 494-501.

281. Sakumoto, R., et al., *Central and peripheral concentrations of tumor necrosis factor-alpha in Chinese Meishan pigs stimulated with lipopolysaccharide.* J Anim Sci, 2003. **81**(5): p. 1274-1280.
282. Escribano, D., et al., *Effect of repeated administration of lipopolysaccharide on inflammatory and stress markers in saliva of growing pigs.* Vet J, 2014. **200**(3): p. 393-397.
283. de Groot, J., et al., *Response to LPS in female offspring from sows treated with cortisol during pregnancy.* Physiol Behav, 2007. **90**(4): p. 612-618.
284. Johnson, R.W. and E. Vonborell, *Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin.* J Anim Sci, 1994. **72**(2): p. 309-314.
285. Falk, M., et al., *Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs.* J Trace Elem Med Biol, 2018. **50**: p. 527-536.
286. de Groot, J., et al., *Response to LPS in female offspring from sows treated with cortisol during pregnancy.* Physiol. Behav., 2007. **90**(4): p. 612-618.
287. Song, C., et al., *Effect of immunological stress to neuroendocrine and gene expression in different swine breeds.* Mol Biol Rep, 2014. **41**(6): p. 3569-76.
288. Tuchscherer, M., et al., *Changes in endocrine and immune responses of neonatal pigs exposed to a psychosocial stressor.* Res Vet Sci, 2009. **87**(3): p. 380-8.
289. Wrona, D., et al., *Stress-induced changes in peripheral natural killer cell cytotoxicity in pigs may not depend on plasma cortisol.* Brain Behav Immun, 2001. **15**(1): p. 54-64.
290. Brandt-Kjelsen, A., et al., *Turnover of Se in adequately fed chickens using Se-75 as a tracer.* J Anim Physiol Anim Nutr (Berl), 2014. **98**(3): p. 547-558.
291. Wahlen, R., *A comparison of GC-ICP-MS and HPLC-ICP-MS for the analysis of organotin compounds.* Agilent technologies, 2002: p. 1-7.
292. Bishop, D.P., et al., *Determination of selenium in serum in the presence of gadolinium with ICP-QQQ-MS.* Analyst, 2015. **140**(8): p. 2842-2846.
293. Michalke, B., *Trace element speciation in human milk.* Pure Appl. Chem., 2006. **78**(1): p. 79-90.
294. Bishop, D.P., et al., *Applications of liquid chromatography-inductively coupled plasma-mass spectrometry in the biosciences: A tutorial review and recent developments.* TrAC Trends in Analytical Chemistry, 2018. **104**: p. 11-21.
295. Mata, J., S. Marguerat, and J. Bähler, *Post-transcriptional control of gene expression: a genome-wide perspective.* Trends in Biochemical Sciences, 2005. **30**(9): p. 506-514.
296. Gry, M., et al., *Correlations between RNA and protein expression profiles in 23 human cell lines.* BMC Genomics, 2009. **10**(1): p. 365.

297. Bustin, S.A., et al., *The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.* Clin Chem, 2009. **55**(4): p. 611-622.
298. Pfaffl, M.W., *A new mathematical model for relative quantification in real-time RT-PCR.* Nucleic Acids Res, 2001. **29**(9): p. e45.
299. Muller, P.Y., et al., *Processing of gene expression data generated by quantitative real-time RT-PCR.* Biotechniques, 2002. **32**(6): p. 1372-4, 1376, 1378-9.
300. Kortner, T.M., et al., *Candidate reference genes for quantitative real-time PCR (qPCR) assays during development of a diet-related enteropathy in Atlantic salmon (Salmo salar L.) and the potential pitfalls of uncritical use of normalization software tools.* Aquaculture, 2011. **318**(3-4): p. 355-363.
301. Fuhrmann, H., H.P. Sallmann, and E. Thesing, *[Effects of vitamins A and E on the antioxidative metabolism of weaning pigs given dietary fats of different qualities].* Dtsch Tierarztl Wochenschr, 1997. **104**(9): p. 387-391.
302. RStudio Team, *RStudio: Integrated Development for R.*, R Foundation for Statistical Computing, Editor. 2016, RStudio, Inc.: Boston, USA.
303. Ingris, *Ingris Årsstatistikk 2011 [Ingris Annual statitics 2011]*. 2012: https://www.animalia.no/contentassets/28e0db72674d496186f0570a9e606fca/arsstatistikk-2011.pdf.
304. Ingris, *Ingris Årsstatistikk 2017 [Ingris Annual statistics 2017]*. 2018: https://www.animalia.no/contentassets/28e0db72674d496186f0570a9e606fca/arsstatistikk_2017.pdf.
305. Yeh, J.Y., et al., *Tissue distribution and influence of selenium status on levels of selenoprotein W.* FASEB J, 1995. **9**(5): p. 392-6.
306. Vendeland, S.C., et al., *Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium.* Proc Natl Acad Sci USA 1995. **92**(19): p. 8749-8753.
307. Yeh, J.Y., et al., *Tissue distribution and influence of selenium status on levels of selenoprotein W.* The FASEB Journal, 1995. **9**(5): p. 392-396.
308. Hoac, T., et al., *Selenoproteins and selenium speciation in food*, in *Selenoproteins and Mimics*. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 183-206.
309. Rehfeldt, C., M. Henning, and I. Fiedler, *Consequences of pig domestication for skeletal muscle growth and cellularity.* Livest Sci, 2008. **116**(1-3): p. 30-41.
310. Koketsu, Y., et al., *Characterization of feed intake patterns during lactation in commercial swine herds.* J Anim Sci, 1996. **74**.
311. Thingnes, S.L., et al., *Ad libitum versus step-up feeding during late lactation: The effect on feed consumption, body composition and production performance in dry fed loose housed sows.* Livest Sci, 2012. **149**(3): p. 250-259.

312. Eissen, J.J., E. Kanis, and B. Kemp, *Sow factors affecting voluntary feed intake during lactation.* Livestock Production Science, 2000. **64**(2–3): p. 147-165.
313. Theil, P., et al., *Lactation, milk and suckling*, in *Nutritional Physiology of Pigs - with emphasis on Danish Production Conditions*, K.E.B. Knudsen, et al., Editors. 2012, Videncenter for Svineproduktion, Landbrug & Fødevarer: Copenhagen, DK. p. 1-49.
314. Zhan, X., et al., *Selenomethionine: an Effective Selenium Source for Sow to Improve Se Distribution, Antioxidant Status, and Growth Performance of Pig Offspring.* Biol. Trace Elem. Res., 2011. **142**(3): p. 481-491.
315. Cao, J., et al., *Effects of dietary selenomethionine supplementation on growth performance, antioxidant status, plasma selenium concentration, and immune function in weaning pigs.* J Anim Sci Biotechnol, 2014. **5**(1): p. 46.
316. Mahan, D.C., et al., *Evaluation of the feeding duration of a phase 1 nursery diet to three-week-old pigs of two weaning weights. NCR-42 Committee on Swine Nutrition.* J Anim Sci, 1998. **76**(2): p. 578-583.
317. Kaushal, N., et al., *The regulation of erythropoiesis by selenium in mice.* Antioxidants & Redox Signaling, 2011. **14**(8): p. 1403-1412.
318. Simard, F., et al., *Effects of concentrations of cyanocobalamin in the gestation diet on some criteria of vitamin B12 metabolism in first-parity sows.* J Anim Sci, 2007. **85**(12): p. 3294-3302.
319. Ballance, D.M. and J.D. House, *Nonruminant nutrition: amino acids and dietary restrictions.* J Anim Sci/J Diary Sci, 2005. **83/88**(Suppl. 1/Suppl. 1): p. 160.
320. Beck, M.A. and O.A. Levander, *Dietary oxidative stress and the potentiation of viral infection.* Annu Rev Nutr, 1998. **18**(1): p. 93-116.
321. Burk, R.F., *Biological activity of selenium.* Annu Rev Nutr, 1983. **3**: p. 53-70.
322. Gross, M., M. Oertel, and J. Köhrle, *Differential selenium-dependent expression of type I 5′-deiodinase and glutathione peroxidase in the porcine epithelial kidney cell line LLC-PK1.* Biochem J, 1995. **306**(3): p. 851-856.
323. Burk, R.F., et al., *Response of rat selenoprotein P to selenium administration and fate of its selenium.* Am J Physiol, 1991. **261**(1 Pt 1): p. E26-E30.
324. Mihara, H., et al., *cDNA cloning, purification, and characterization of mouse liver selenocysteine lyase: Candidate for selenium delivery protein in selenoprotein synthesis.* J Biol Chem, 2000. **275**(9): p. 6195-6200.
325. Sunde, R.A., et al., *Selenium regulation of selenium-dependent glutathione peroxidases in animals and transfected CHO cells.* Biomed Environ Sci, 1997. **10**(2-3): p. 346-355.

326. Schomburg, L., U. Schweizer, and J. Köhrle, *Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic.* Cell Mol Life Sci, 2004. **61**(16): p. 1988-1995.
327. Devillers, N., J. Le Dividich, and A. Prunier, *Influence of colostrum intake on piglet survival and immunity.* Animal, 2011. **5**(10): p. 1605-1612.
328. Novák, L., L. Kukla, and L. Zeman, *Characteristic differences between the growth of man and other animals.* Prague Med Rep, 2007. **108**: p. 155-166.
329. Ballerini, A., et al., *Traceability of inbred and crossbred Cinta Senese pigs by evaluating the oxidative stress.* J Vet Med A Physiol Pathol Clin Med, 2003. **50**(3): p. 113-116.
330. Knap, P. and W.M. Rauw, *Selection for high production in pigs*, in *Resource Allocation Theory Applied to Farm Animal Production*, W.M. Rauw, Editor. 2008, CABI Publishing. p. 210-229.
331. Babicz, M., et al., *Analysis of relationship between sows colostrum and milk somatic cell count and reproductive performance traits.* Ann Univ Mariae Curie Skłodowska Sect EE Zootech, 2011. **3**(29): p. 1-13.
332. Csapó, J., et al., *Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days.* Int Dairy J, 1996. **6**(8): p. 881-902.
333. Zou, S., D.G. Mclaren, and W.L. Hurley, *Pig colostrum and milk-composition - Comparisons between Chinese Meishan and United-States breeds.* Livest Prod Sci, 1992. **30**(1-2): p. 115-127.
334. Stelwagen, K. and K. Singh, *The role of tight junctions in mammary gland function.* J Mammary Gland Biol Neoplasia, 2014. **19**(1): p. 131-8.
335. Mou, D., et al., *Effect of maternal organic selenium supplementation during pregnancy on sow reproductive performance and long-term effect on their progeny.* J Anim Sci, 2020. **98**(12).
336. Deagen, J.T., et al., *Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma.* Anal Biochem, 1993. **208**(1): p. 176-181.
337. Burk, R.F. and K.E. Hill, *Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis.* Annual Review of Nutrition, 2005. **25**(1): p. 215-235.
338. Kasik, J.W. and E.J. Rice, *Selenoprotein p expression in liver, uterus and placenta during late pregnancy.* Placenta, 1995. **16**(1): p. 67-74.
339. Matte, J.J. and C.L. Girard, *Changes of serum and blood volumes during gestation and lactation in multiparous sows.* Can. J. Anim. Sci., 1996. **76**: p. 263-266.
340. Burk, R.F., et al., *Maternal-fetal transfer of selenium in the mouse.* FASEB J, 2013. **27**(8): p. 3249-3256.
341. Chase, P.H., J.J. Volpe, and L. Laster, *Transsulfuration in mammals: fetal and early development of methionine-activating enzyme and its*

relation to hormonal influences. J Clin Invest, 1968. **47**(9): p. 2099-2108.
342. Far, J., H. Preud'homme, and R. Lobinski, *Detection and identification of hydrophilic selenium compounds in selenium-rich yeast by size exclusion–microbore normal-phase HPLC with the on-line ICP–MS and electrospray Q-TOF-MS detection.* Anal Chim Acta, 2010. **657**(2): p. 175-190.
343. Nakagaki, B.N., et al., *Immune and metabolic shifts during neonatal development reprogram liver identity and function.* Journal of Hepatology, 2018. **69**(6): p. 1294-1307.
344. Mahan, D.C. and Y.Y. Kim, *Effect of inorganic or organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first-parity gilts and their progeny.* J Anim Sci, 1996. **74**(11): p. 2711-2718.
345. Olson, G.E., et al., *Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells.* J. Biol. Chem., 2008. **283**(11): p. 6854-6860.
346. Berry, E.J., E.C. Segerson, and D.W. Libby, *Serum and testis selenium concentrations and testis morphology in ram lambs of varying ages.* Theriogenology, 1983. **20**(4): p. 473-483.
347. Behne, D., M. Duk, and W. Elger, *Selenium content and glutathione peroxidase activity in the testis of the maturing rat.* J Nutr, 1986. **116**(8): p. 1442-1447.
348. Dalto, D.B., et al., *The importance of pyridoxine for the impact of the dietary selenium sources on redox balance, embryo development, and reproductive performance in gilts.* J Trace Elem Med Biol, 2016. **34**: p. 79-89.
349. Lu, J., C. Berndt, and A. Holmgren, *Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase.* Biochim. Biophys. Acta, 2009. **1790**(11): p. 1513-9.
350. Spyrou, G., et al., *Selenite and selenate inhibit human lymphocyte growth via different mechanisms.* Cancer Research, 1996. **56**(19): p. 4407-4412.
351. Guan, L., et al., *Sodium selenite induces apoptosis by ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction in human acute promyelocytic leukemia NB4 cells.* Apoptosis, 2009. **14**(2): p. 218-225.
352. Palsamy, P., K.R. Bidasee, and T. Shinohara, *Selenite cataracts: activation of endoplasmic reticulum stress and loss of Nrf2/Keap1-dependent stress protection.* Biochim Biophys Acta, 2014. **1842**(9): p. 1794-1805.
353. Ye, Y., et al., *A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol.* Nature, 2004. **429**(6994): p. 841-847.
354. Marino, M., et al., *SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts*

hyperoxidation by means of redox-regulating SERCA2 pump activity. Hum Mol Genet, 2015. **24**(7): p. 1843-1855.
355. Gao, Y., et al., *Regulation of the selenoprotein SelS by glucose deprivation and endoplasmic reticulum stress - SelS is a novel glucose-regulated protein.* FEBS Lett, 2004. **563**(1-3): p. 185-190.
356. Petit, N., et al., *Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern.* Hum Mol Gen, 2003. **12**(9): p. 1045-1053.
357. Yeh, J.-Y., et al., *Selenium influences tissue levels of selenoprotein W in sheep.* J Nutr, 1997. **127**(3): p. 394-402.
358. Yeh, J.-Y., et al., *Dietary selenium increases selenoprotein W levels in rat tissues.* J Nutr, 1997. **127**(11): p. 2165-2172.
359. Whanger, P.D., *Selenoprotein expression and function-selenoprotein W.* Biochim. Biophys. Acta, 2009. **1790**(11): p. 1448-52.
360. Whanger, P.D., *Selenoprotein W: a review.* Cell Mol Life Sci, 2000. **57**(13-14): p. 1846-1852.
361. Sun, Y., et al., *Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of the rat.* J Nutr Biochem, 1998. **9**(1): p. 23-27.
362. Yao, H., et al., *Selenoprotein W redox-regulated Ca2+ channels correlate with selenium deficiency-induced muscles Ca2+ leak.* Oncotarget, 2016. **7**(36): p. 57618-57632.
363. Suzuki, K.T., *Metabolomics of selenium: Se metabolites based on speciation studies.* J. Health Sci., 2005. **51**(2): p. 107-114.
364. Daiwen, C., Z. Keying, and W. Chunyan, *Influences of lipopolysaccharide-induced immune challenge on performance and whole-body protein turnover in weanling pigs.* Livestock Science, 2008. **113**(2): p. 291-295.
365. Minnich, V., et al., *Glutathione biosynthesis in human erythrocytes. I. Identification of the enzymes of glutathione synthesis in hemolysates.* J Clin Invest, 1971. **50**(3): p. 507-513.
366. Yan, L., L.M. Boylan, and J.E. Spallholz, *Effect of dietary selenium and magnesium on human mammary tumor growth in athymic nude mice.* Nutr Cancer, 1991. **16**(3-4): p. 239-248.
367. Hill, K.E. and R.F. Burk, *Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes.* J Biol Chem, 1982. **257**(18): p. 10668-10672.
368. Lauridsen, C., et al., *Lactating sows and suckling piglets preferentially incorporate RRR- over all-rac-α-tocopherol into milk, plasma and tissues.* The Journal of Nutrition, 2002. **132**(6): p. 1258-1264.
369. Shurson, J., et al. *Vitamin E and selenium status of pigs fed DDGS diets and relationship to Mulberry Heart Disease*. 2013. 22 DOI: http://www.auri.org/assets/2013/01/Vitamin-E-and-selenium-status-of-pigs-fed-DDGS-diets-and-relationship-to-Mulberry-Heart-Disease1.pdf.

370. Zurawska-Plaksej, E., et al., *Oxidatively modified forms of albumin in patients with risk factors of metabolic syndrome.* J Endocrinol Invest, 2014. **37**(9): p. 819-827.
371. Figueroa, J., et al., *Anhedonia in pigs? Effects of social stress and restraint stress on sucrose preference.* Physiology & Behavior, 2015. **151**: p. 509-515.
372. Pluske, J.R., D.J. Hampson, and I.H. Williams, *Factors influencing the structure and function of the small intestine in the weaned pig: a review.* Livestock Production Science, 1997. **51**(1): p. 215-236.
373. Moeser, A.J., et al., *Defects in small intestinal epithelial barrier function and morphology associated with peri-weaning failure to thrive syndrome (PFTS) in swine.* Research in Veterinary Science, 2012. **93**(2): p. 975-982.
374. Robinson, W.F. and N.A. Robinson, *Cardiovascular system*, in *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. 2015. p. 1-101.
375. Thorn, C.E., *Hematology of the pig*, in *Schalm's Veterinary Hematology, 6th Edition*, D.J. Weiss and K.J. Wardrop, Editors. 2010, Wiley-Blackwell: Ames, Iowa. p. 843-851.
376. Bhattarai, S. and J.P. Nielsen, *Association between hematological status at weaning and weight gain post-weaning in piglets.* Livest Sci, 2015. **182**: p. 64-68.
377. Rootwelt, V., et al., *Postpartum deaths: piglet, placental, and umbilical characteristics.* J Anim Sci, 2013. **91**(6): p. 2647-2656.
378. Sun, Y., et al., *Vitamin E supplementation protects erythrocyte membranes from oxidative stress in healthy Chinese middle-aged and elderly people.* Nutr Res, 2012. **32**(5): p. 328-334.

Enclosed papers I-IV

Paper I

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Porcine Health Management

CASE REPORT Open Access

A descriptive report of the selenium distribution in tissues from pigs with mulberry heart disease (MHD)

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Abstract

Background: Mulberry Heart Disease (MHD) is a condition affecting mainly young pigs in excellent body condition. Feed efficient pigs showing high average daily gains are more likely to be affected. MHD has been described as a challenge in Norwegian pig production over the last decade despite abundant supplies of vitamin E, and selenium (Se) close to the upper limits set by the EU. From 2015 to 2017, samples from documented MHD field cases were collected and compared with controls regarding post mortem findings and Se concentrations in numerous internal and external organs were determined in order to characterize the Se distribution, and to identify any differences between MHD cases and controls.

Case presentation: Eight MHD cases from commercial farms and a pet pig producer located in the South West and East of Norway, and three control animals originating from these farms were included in this study. MHD cases and controls were weaned pigs with an average bodyweight (BW) of 17 kg (range 9 to 46 kg BW), with the exception of one pet piglet (Mangalica, 6 kg BW) that had only received sow milk. Selenium was determined in samples from the cardiovascular, digestive, immune, endocrine, integumentary, muscular, respiratory and urinary systems using inductively coupled plasma mass spectrometry (QQQ ICP-MS). All pigs with MHD suffered sudden deaths. Control animals were euthanized without being bled prior to necropsy and sampling. Significantly different mean Se concentrations between MHD cases and controls were found in cardiac samples as well as almost all skeletal muscles ($P < 0.05$). Based on the samples from ten different muscles (except the cardiac samples), mean Se concentrations in MHD cases were 0.34 (0.01) mg/ kg DM compared with 0.65 (0.02) mg/ kg DM in control pigs ($P < 0.0001$). In cardiac samples, mean Se concentrations from MHD cases were 0.87 (0.02) mg/ kg DM vs. 1.12 (0.04) mg/ kg DM ($P < 0.0001$). Additionally, significantly lower Se concentrations compared with controls were found in the liver as well as the caecum, duodenum, gastric ventricle, jejunum, kidney, skin and thymus samples.

Conclusions: Based on the present work, the current common practice regarding tissue analyses in MHD cases could be refined to include other organs than liver and heart. The evident differences in mean Se concentrations in 9 out of 10 samples from the muscular system, could make such samples relevant for complementary measurements of Se concentrations to help confirm the MHD diagnosis. We find it interesting that although our limited number of sampled pigs are different in terms of genetics, size and feeding regimes, the variation of Se concentrations in a given organ was low between MHD cases. Since this report includes a limited number of MHD cases and controls, our results should be corroborated by a controlled, larger study.

Keywords: Mulberry heart disease, Skeletal muscle, Liver, Cardiac, Muscular, Gastrointestinal, Selenium, Distribution

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Background

Selenium (Se) deficiency involved in fatal cardiomyopathy is well known in pigs [1–3]. Se deficiency probably causes uncompensated oxidative stress leading to cellular damage, often resulting in death [3]. The importance of Se and selenoproteins in muscle tissue physiology is well documented. Studies have shown that the gene expression, plasma and tissue concentrations of selenoprotein W and selenoprotein P as well as glutathione peroxidase activity, are higher in Se-supplemented than Se-deficient animals [4–8].

Mulberry heart disease (MHD) is a peracute to acute condition, appearing mainly in pigs of two to four months of age. It has, however, been observed in pigs as young as three weeks. Typically pigs in excellent body condition are found dead. The principal gross lesions in pigs succumbing to MHD are straw-colored fluid in the pleural cavity, transudate with fibrin in the pericardium and edematous lungs. The myocardium appears mottled due to transmural hemorrhage and pale necrotic areas. Oxidative stress causes oxidative modifications of myofilament proteins like actin, titin and myosin and can thereby impair the contractility of myocytes [9]. The above mentioned alterations in myocytes combined with macroscopic and microscopic lesions of MHD cases strongly suggest ventricular dysrhythmia followed by acute heart failure. The diagnosis of MHD can be confirmed when the following microscopic heart lesions are observed: In acute cases interstitial hemorrhage is the main lesion, whereas in less acute cases degeneration and necrosis of myofibers, sometimes with mineralization, are observed. [10].

Tremendous advancement within molecular biology and genomics over the last decades, e.g. the sequencing of the porcine genome [11], has facilitated development and improvement of sophisticated research methods and technology. The added value of DNA information to breeding values can now contribute to rapid genetic progress [12]. The breeding goals of most pig breeding companies globally include lean growth efficiency, reduced feed intake per kg growth and reduced backfat [13–17].

The number of publications on MHD and Se concentrations in porcine tissues in peer reviewed literature is limited, and the majority of the existing reports are more than fifteen years old [10, 18–21]. Some of these reports have stated that tissue concentrations of Se in heart and liver samples are within the normal range in MHD cases [10, 18, 19]. Unpublished cases from Norway over the last five years support these statements, also with results based on Se concentrations in liver samples. Nevertheless MHD cases cease to occur in affected herds after Se supplementation, either by injection treatments with Se or feed additives containing Se and vitamin E.

This study was carried out on MHD cases and control pigs reared under field conditions, in order to characterize the Se distribution in multiple tissues of MHD cases and controls prior to and after weaning. To the authors' knowledge, this is the first report to describe the Se distribution in numerous internal and external organs of pigs diagnosed with MHD.

Case presentation

The pigs included in this study were submitted for post mortem examinations to the Norwegian Veterinary Institute in Oslo and Sandnes and the Norwegian University of Life Sciences in Sandnes between 2015 and 2017. The pigs originated from five commercial piglet producing farms (Farms 1 to 5) and one pet pig producer (Farm 6) located in the counties of Oppland and Rogaland in Norway (Table 1). The piglet from the latter farm had only been fed sows' milk.

The Se added to the compound feedingstuffs provided at the farms was sodium selenite at levels between 0.33 mg/ kg and 0.40 mg/ kg feed. The amount of alpha-tocopherol (vit E) added to the feed was between 100 mg/ kg to 200 mg/kg feed. Non-supplemented raw materials used to produce compound feedingstuffs for pigs with Norwegian origin contain negligible Se levels around 0.05 mg/ kg [4]. The feed composition of the feed provided at the different farms is listed in Table 2.

MHD cases and control pigs underwent complete post mortem examinations. Pigs included as MHD cases had died suddenly and showed both typical macroscopic (Fig. 1) and (Fig. 2) microscopic lesions. Control pigs were selected from two of the farms submitting MHD cases. These pigs had the same genetic background and similar size as the confirmed MHD cases. Control animals were pigs showing good average daily gain compared to their littermates and other litters of the same batch, and they had not been treated due to sickness. Control animals showed no signs of pathological lesions when undergoing post mortem examinations.

A sampling protocol was elaborated to ensure a standardized sampling procedure for each organ. Tissue samples from the cardiovascular, digestive, immune, endocrine, integumentary, nervous, muscular, reproductive, respiratory and urinary systems were obtained (Table 3). Samples were dried with paper sheets to remove surplus blood. Intestinal samples were rinsed with water to remove intestinal contents prior to drying off with paper.

From all animals, tissue samples from the myocardium were examined histologically. Additionally, samples from tissues showing lesions were examined. Tissue samples of approximately 15 × 10 × 5 mm were fixed in 4% formaldehyde for one week and then dehydrated in graded ethanols and paraffin embedded. Sections (4 μm) were mounted on slides and stained with hematoxylin and

Table 1 Description of included Mulberry Heart Disease (MHD) and control pigs in the case study

No.	MHD/ Control	Farm	Sex	Age (weeks)	Bodyweight (kg)	Genetics	Iron treatment*	Sodium selenite (mg/ kg)	Alpha-tocopherol (mg/ kg)
1	MHD	1	C	14	45.0	HHZL	Oral paste/ iron-enriched peat	0.40	175.00
2	MHD	2	F	6	12.0	HHZL	Injection	0.39	180.00
3	MHD	2	C	6	16.0	HHZL	Injection	0.39	180.00
4	MHD	3	C	6	11.0	LLLL	Injection	0.33	100.00
5	MHD	3	F	6	10.0	LLLL	Injection	0.33	100.00
6	MHD	4	C	6	9.0	DDLL	Oral paste/ iron-enriched peat	0.40	162.00
7	MHD	5	C	8	14.0	DDZL	Oral paste/ iron-enriched peat	0.33	100.00
8	MHD	6	F	6	6.0	Mangalica	Injection	Sow milk	
1	Control	1	C	14	42.0	HHZL	Iron paste and	0.40	175.00
2	Control	2	C	6	18.0	HHZL	Iron injection	0.39	180.00
3	Control	2	F	7	25.0	HHZL	Iron injection	0.39	180.00

Female (F) and castrated (C) pigs in this study originated from five commercial pig producing farms and one pet farmer. The pigs included in this study were both male and female pigs between six and fourteen weeks old. The Se source used in the complete feed was sodium selenite (NaSe) and added levels of NaSe were between 0.33 and 0.40 mg/ kg feed. The vitamin E (vit E) source was alpha tocopherol and added levels were between 100.00 and 200.00 mg/ kg feed. The grower-finisher pigs from Farm 1 were fed liquid feed in combination with whey from approximately 30 kg bodyweight or approximately ten weeks of age. *Iron treatment of the piglets was applied within the first four days of life, either by injecting 200 mg subcutaneously or providing 300 mg of bioavailable iron per os combined with iron-enriched peat until weaning

eosin (HE). Light microscopic examination was conducted to specifically assess for myocardial lesions compatible with MHD. Pigs with characteristic macroscopic lesions combined with the following myocardial histopathology were considered MHD cases: Severe subepicardial and myocardial hemorrhages, swollen cardiac myofibers with loss of cross striations, and hypereosinophilic myofibers with pyknotic nuclei.

Samples of different tissues were collected at the same localization from each animal, both MHD cases and controls. Approximately 50 mg of tissue for ICP-MS analysis were placed in 1.8 mL cryotubes (Nunc Cryotube™, Sigma-Aldrich, Leirdal, Norway) and stored at − 20 °C until ICP-MS analysis. From the cardiac muscle, transmural samples were obtained from the center of the right and left free ventricular wall. Additionally, a transmural sample from the center of the septum was obtained. The gastric ventricle was sampled at the *Curvatura major*. Samples from the gastrointestinal tract were isolated from the proximal section of each intestinal region. From the liver, tissue was sampled from the lobe adjacent to the gallbladder. The splenic lobe of the pancreas was sampled. Both parathyroid glands were collected. Samples from the spleen, thymus, thyroid gland, skeletal muscles and kidney were obtained from the center of the organs. The lung was sampled from the right or left caudal lobes. The skin and bristles were sampled from the left or right lateral abdominal flank. Claw samples were from the distal tip of the left or right hoof wall.

A total of five to eight samples per tissue were analyzed for their total Se concentration (MHD cases) and compared to Se levels in samples from the three control animals. The concentrations of Se in collected organs were determined using inductively coupled plasma mass spectrometry (Agilent 8800 QQQ ICP-MS, Japan) at the Norwegian University of Life Sciences (NMBU/MINA). The organs were weighed, freeze-dried, transferred to acid cleaned Teflon tubes, and then weighed once more. The samples were added 2 ml water, 60 ng 74Se (enriched to 99.9%) as internal standard and 1.5 mL conc HNO3 (ultrapure quality). The samples, CRM and blanks were digested in an UltraClave and/or UltraWave from Milestone at 260 °C for about 20 min. After digestion, all samples were diluted to 15 ml prior to measurements [22]. Bovine Liver 1577c served as CRM. Limit of detection (LOD) and limit of quantification (LOQ) were calculated, 3 and 10 times respectively the standard deviation of the method blanks. In the present work LOD 0.005 mg Se/kg, LOQ 0.016 mg Se/kg.

Microbiologic examination was conducted on selected tissues from all animals. Specimens were inoculated on sheep blood agar for 48 h at 37 °C and 5% CO_2. No bacterial growth was identified from any of the specimens tested.

All data were exported to Excel (Microsoft Corporation, Redmond, Washington) and then imported into JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC 2751, USA) for statistical analyses. Normality of data was tested by the Shapiro-Wilk test and homogeneity of variance. Differences between groups were analyzed by 1-way analysis of variance with concentrations of Se as the dependent variable and the animals' status (MHD or control) as the independent variable. The farm, feed, sex and age were used as covariates. The p-level was set to 0.05. Potential outliers were

Table 2 Feed composition

%	Farm 1*		Farm 2		Farm 3	Farm 4	Farm 5	Farm 6
	MHD1	C1	MHD2/ MHD3	C2/ C3	MHD4 MHD5	MHD6	MHD7	MHD8
Fishmeal LT-94			7.00	7.00		5.00		Sow milk
Barley	35.48	32.16	16.80	10.00	43.13	22.60	27.71	
Soybean cake flour						10.00	8.00	
Soybean meal	13.28	13.21	6.00	4.00	14.20	1.60		
Soybean meal Hipro				6.00			11.00	
Soybean oil	0.38		0.60	1.40	1.00	0.50	1.00	
Wheat	5.00	6.00	50.24	45.71	10.00	49.89	15.00	
Rapeseed cake	8.00	8.00			8.00			
Oats	25.00	30.00	1.70	3.00	9.50		15.00	
Pea starch	5.00		3.00	3.00	5.00		6.20	
Limestone	1.27	1.29						
Animal fat	2.00	4.03	3.20	2.10	3.00	0.70	3.00	
Field beans	5.00	2.00	3.00	3.00		5.00	7.00	
Molasses sugar cane			0.70	0.50	2.00	1.00	2.00	
Sunflower cake			2.30	0.60				
Corn gluten			2.00	0.80				
Corn grits				10.00				
Mikromin Pig[a]	0.23	0.23						
Vitamin ADKB[b]	0.08	0.08	0.08	0.08	0.06	0.08	0.06	
mg/ kg								
Sodium selenite (NaSe)	0.40	0.40	0.39	0.39	0.33	0.40	0.33	
Alpha-tocopherol	175.00	175.00	180.00	180.00	100.00	162.00	100.00	

The feed composition of the feed provided both MHD cases and controls is listed
[a,b] Additives containing the following per kilogram of diet:
[a] Fe 96 mg; Cu 20.8 mg; Mn 48 mg; Zn 96 mg; I 0.48 mg
[b]Vitamin A 5700 IU; Vitamin D 1200 IU; Vitamin E
100 mg; Vitamin K 3.72 mg; Vitamin B1 2.4 mg; Vitamin B2
4.5 mg; Vitamin B5 12.0 mg; Vitamin B6 7.2 mg; Vitamin
B12 0.012 mg; Folic acid 1.8 mg; Biotin 0.24 mg
*At Farm 1, the feed was supplemented with whey, accounting for 25% of the total energy

assessed graphically and model diagnostics were performed.

In all MHD cases, the diagnosis was confirmed by typical macroscopic lesions and histopathological findings. All MHD cases had straw-colored transudate in the pleural and pericardial cavity, often with fibrin strands (Fig. 1). Additionally, pulmonary edema as well as pale and reddened areas of the myocardium due to subepicardial (Fig. 2) and myocardial hemorrhages (Fig. 3) were found. Microscopically, interstitial hemorrhage was observed along with swollen cardiac myofibers that had lost cross striations (Fig. 4). Some histological sections also showed microthrombi, degenerative and necrotic areas with local mineralization.

No lesions were found in skeletal muscles or liver tissues, except MHD case no. 8, also showing macro- and microscopic lesions compatible with *Hepatosis dietetica*.

Based on all ten skeletal muscular samples, mean Se concentrations in MHD cases were 0.34 (0.01) mg/ kg compared with 0.65 (0.02) mg/ kg in control pigs ($P < 0.0001$). Samples from the cardiovascular system showed significantly lower mean Se concentrations in all sampled areas of MHD cases compared with control pigs. Greater difference in mean Se concentrations was observed in the left ventricular wall of the heart; 0.84 (0.15) mg/ kg vs. 1.13 (0.12) mg/ kg ($P = 0.0070$, Table 3). If combining the results from the three cardiac samples, the mean Se concentration in MHD cases was 0.87 (0.02) mg/ kg as opposed to 1.12 (0.04) mg/ kg ($P < 0.0001$) in the cardiac samples from controls.

Within the digestive system, lower average Se concentrations were found in samples from the MHD cases' gastric ventricle, duodenum, jejunum and caecum. In the liver samples, mean Se concentrations of MHD cases were 1.29 (0.20) mg/ kg vs 1.77 mg/kg (0.40) in controls

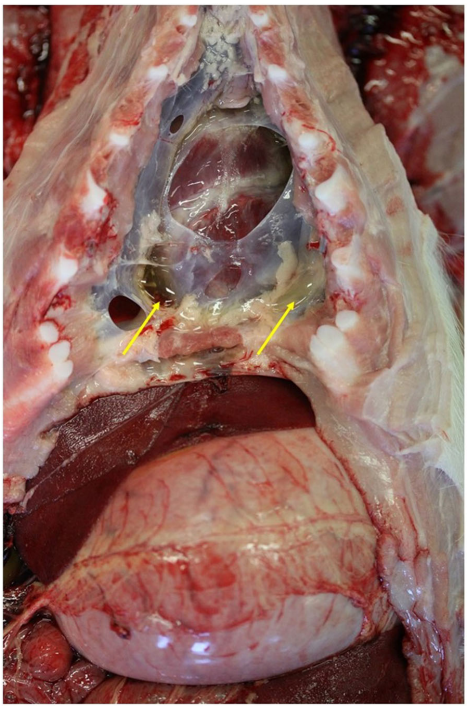

Fig. 1 Transudate in the pleural cavity

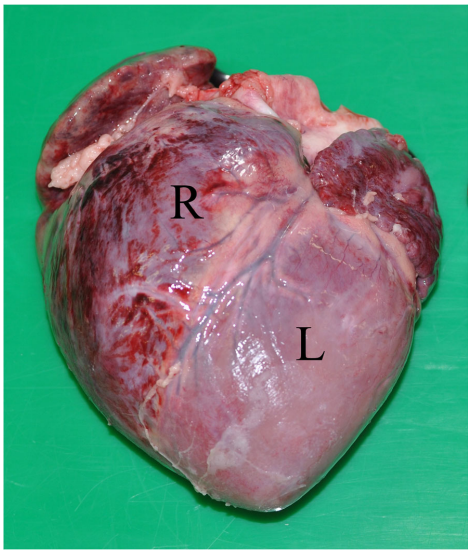

Fig. 2 Subepicardial hemorrhages

($P = 0.020$). No differences between MHD cases and controls were found in samples from the adrenal glands, claws, colon, ileum, lungs, lymph nodes, pancreas, parathyroid gland, spleen or thyroid gland.

Discussion and conclusions

The sampled pigs in this report constitute a heterogenous group since multiple genetic lines fed different feed and pigs with differing bodyweight are represented. These are factors potentially influencing the pigs' susceptibility to oxidative stress and MHD. We find it interesting that although our limited number of sampled pigs are different in terms of genetics, size and feeding regimes, the variation of Se concentrations in a given organ is low between MHD cases.

The results from this study provide information about the Se distribution both in internal and external organs in MHD cases and designates muscle tissue as particularly interesting regarding the diagnostic approach. Most reports describing pigs with MHD refer to vitamin E and Se concentrations in liver samples [10, 18–20, 23]. Although biodilution cannot be excluded (difference in weight and age), our results did render significant differences in liver Se concentrations between MHD cases and control animals. Mean liver concentrations of MHD cases (dry basis) in this study were 1.29 mg/kg (0.20). A liver concentration of 1.2 mg Se/ kg (dry basis) was suggested by Lindberg and Siren as normal [24]. Other studies have stated that MHD cases apparently show Se liver concentrations within what is considered the normal range [10, 19]. Thus, the questions arise if the Se concentrations in liver samples from healthy, high-yielding pigs of today should actually be higher, if other samples should be collected and analyzed, and if genetic differences between breeds could affect the actual Se requirements. A recent study has shed light on the micronutrient-genetic relationships and showed that genetic background can affect the intake of minerals [25]. Typically MHD cases are pigs growing rapidly. This feature combined with possible individual disparities regarding feed intake and efficiency may contribute to a disruption in proper mineral intake and cause certain individuals within a group of pigs to succumb to MHD.

Most previous reports on MHD also include Se concentrations in myocardial samples, which is reasonable since pathological findings are found invariably in the myocardium of MHD cases. Here, a significant difference was found between MHD samples and controls

Table 3 Se concentrations in different organ samples from cases of Mulberry Heart Disease (MHD) and controls (mg/ kg dry weight)

Organ system	Organ samples	MHD		Control		*P*-value
		Mean Se (mg/ kg)	n	Mean Se (mg/ kg)	n	
Cardiovascular system	Myocardium, left ventricle	0.84 (0.15)	8	1.13 (0.12)	3	0.0070
	Myocardium, right ventricle	0.92 (0.04)	5	1.11 (0.15)	3	0.032
	Myocardium, septum	0.88 (0.04)	6	1.16 (0.06)	3	0.0059
Digestive system	Caecum	0.63 (0.11)	8	0.87 (0.18)	3	0.020
	Colon	0.77 (0.19)	8	1,04 (0.23)	3	ns
	Duodenum	0.78 (0.15)	7	1.20 (0.10)	3	0.0020
	Gastric ventricle	0.64 (0.13)	6	0.93 (0.08)	3	0.0090
	Ileum	0.86 (0.11)	8	1.04 (0.20)	3	ns
	Jejunum	0.90 (0.14)	8	1.27 (0.15)	3	0.0040
	Liver	1.29 (0.20)	8	1.77 (0.40)	3	0.020
	Pancreas	1.01 (0.28)	7	1.18 (0.57)	3	ns
Immune and endocrine system	*Ln ileocolici*	1.07 (0.42)	8	1.43 (0.16)	3	ns
	Ln poplitei	0.78 (0.27)	8	0.70 (0.13)	3	ns
	Parathyroid gland	0.88 (0.33)	5	0.80 (0.16)	3	ns
	Spleen	1.18 (0.17)	5	1.33 (0.06)	3	ns
	Thymus	0.97 (0.06)	8	1.16 (0.15)	3	0.012
	Thyroid gland	0.64 (0.13)	7	0.79 (0.12)	3	ns
	Adrenal gland	1.20 (0.08)	5	1.20 (0.10)	3	ns
Integumentary	Claw	0.41 (0.12)	5	0.66 (0.25)	3	ns
	Skin	0.14 (0.02)	7	0.25 (0.07)	3	0.0041
Muscular system	Diaphragm	0.39 (0.10)	6	0.62 (0.11)	3	0.020
	M. biceps brachii	0.35 (0.09)	8	0.64 (0.17)	3	0.0040
	M. extensor carpi radialis	0.34 (0.07)	8	0.66 (0.19)	3	0.0020
	M. extensor digitorum longus	0.33 (0.07)	8	0.65 (0.17)	3	0.0010
	M. longissimus dorsi lumbalis	0.35 (0.05)	5	0.62 (0.24)	3	0.042
	M. longissimus dorsi thobaracis	0.43 (0.23)	8	0.66 (0.25)	3	ns
	M. psoas major	0.34 (0.08)	8	0.67 (0.21)	3	0.0028
	M. quadriceps femoris	0.33 (0.08)	7	0.68 (0.22)	3	0.0040
	M. semimembranosus	0.32 (0.08)	8	0.64 (0.18)	3	0.0018
	M. semitendinosus	0.28 (0.05)	8	0.62 (0.20)	3	0.0011
Respiratory system	Lung	0.96 (0.23)	8	1.23 (0.06)	3	ns
Urinary system	Kidney	4.93 (0.57)	7	6.23 (1.07)	3	0.030

Se concentrations in internal and external samples from MHD and control pigs. Results are based on dry weight analyses

with respect to the samples isolated from the septum ($P < 0.01$), left ($P < 0.01$) and right ventricle ($P < 0.05$) of the myocardium. This is not in accordance with previous studies, reporting *no* difference between MHD cases and controls in terms of Se concentrations in cardiac samples [18, 26]. In a recent publication the authors stated that different muscle tissues have distinct intrinsic mitochondrial respiratory functions, which likely influences the efficiency of oxidative phosphorylation and could potentially alter reactive oxygen species (ROS) production [27] .The cardiac muscle with its high metabolic demand is rich in mitochondria, accounting for approximately 35% of the cardiac tissue volume. Skeletal muscles exhibit approximately half of the mitochondrial density found in the cardiac muscle. Since mitochondria are the most important cellular source of ROS [28], this may contribute to the cardiac muscle susceptibility to ROS-induced oxidative injury if a pig is deficient in antioxidant factors. A rodent Se deficiency and repletion model showed a distinct distribution of selenoenzymes,

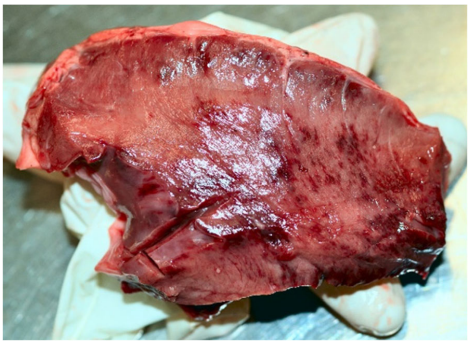

Fig. 3 Myocardial hemorrhages

suggesting that the heart may be the organ most sensitive to oxidative stress [29]. The fact that all MHD cases showed macroscopic and microscopic lesions in cardiac samples but not in skeletal muscles may support this theory. Within the cardiac muscle, some areas may be of particular importance for sampling, like the papillary muscles. They are located in both ventricles of the heart and it has been shown that papillary muscles are prone to fibrosis upon oxidative stress insults [30]. A more standardized approach for sampling of the cardiac muscle of MHD cases may contribute to less interindividual variability regarding Se concentrations.

In industrialized pork production, feed costs account for approximately 60 to 70% of the total production costs [16, 17, 31, 32]. Since higher economic outputs of pork production can be achieved by improved feed efficiency, selection for high lean growth rate and reduced backfat, commercial pig lines have been systematically bred over decades to improve these traits [15, 33, 34]. Norsvin is a breeding company owned by Norwegian pig producers with a research department at the Norwegian University of Life Sciences (NMBU). The breeding program pursued by Norsvin has e.g. led to a reduction in necessary feed per kg of weight gain and increased average daily gain (ADG) in weaned pigs and grower-finishers over the last ten years [35, 36]. In 2007, Norwegian conventional weaned pigs (typically DLYL, LLLL or YYLL) between approximately 10 and 30 kg live weight (LW) showed ADG of 489 g vs. 582 g in 2017. Grower-finishers between approximately 30 kg and 115 kg LW showed an ADG of 955 g in 2007 vs. 1018 g in 2017 (Table 4). During the same time period (2007 to 2017), the amount of feed per unit gain was reduced in both weaned pigs (1.81 to 1.71) and grower-finishers (2.74 to 2.68). The lean meat percentage in finisher pigs rose from 56.5 to 59.8%. Typically, Norwegian pig feed is added between 0.35 and 0.40 mg Se/ FU, both for weaned and grower-finisher pigs (until recently the dominating Se source has been inorganic sodium selenite). This means that during the last decade, there has been a reduction in available Se. This is due to the reduced amount of necessary feed per unit weight gain and the concurrent increase in body protein deposition, possibly contributing to the occurrence of MHD.

Several authors have considered the theory of resource allocation in lean and feed efficient pigs, which is built on the assumption that different biological processes require different nutritional resources [37–40]. The systematic selection for leaner pigs over decades may have led to a trade-off towards growth in situations where nutritional resources are scarce. Processes involving e.g. the immune and antioxidative status of the animal can thereby be weakened and result in pigs more susceptible to oxidative assaults. Our results showing significantly lower Se concentrations in samples from the thymus of MHD cases may strengthen this theory.

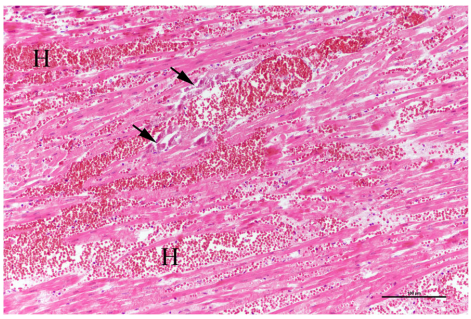

Fig. 4 Interstitial hemorrhages

Table 4 Development of Norwegian pig production results from 2007 to 2017

Production trait	Age group	Year	
		2007	2017
Average daily weight gain (g/ day)	Weaned pigs	489	582
	Grower-finisher pigs	955	1018
Feed units per kg gain (FU/ kg)	Weaned pigs	1.81	1.71
	Grower-finisher pigs	2.74	2.68
Lean meat percentage (%)	Grower-finisher pigs	56.50	59.80

The numbers origin from the annual Ingris report, an online tool available for both Norwegian breeding (nucleus and multiplier) herds as well as piglet producing and finisher units. In 2007, 35% of sow farms and 3.1% of finisher farms were represented in the Ingris annual report. In 2017, 54% of sow farms and 12.3% of finisher farms were represented. In the Norwegian pig production system, weaned pigs are between approximately 10 kg LW and 30 kg LW. Grower-finishers are between approximately 30 kg LW and 115 kg LW

The MHD cases showed significantly lower mean Se concentrations in the samples from the gastric ventricle ($P < 0.01$), the duodenum ($P < 0.005$), jejunum ($P < 0.005$) and caecum ($P < 0.05$). These findings may partly be explained by the absorption pattern of Se in the intestinal tract. In swine, more Se is absorbed in the last part of the small intestine, cecum and colon than in the stomach and proximal parts of the small intestine [41]. Oxidative stress is a major cause of gastrointestinal (GI) damage [42] and robust immunologic mechanisms are required to protect the mucosal surface. The Se dependent glutathione peroxidase GSHPx-GI appears to be the major glutathione-dependent peroxidase in the GI tract and this molecule could play a major role in protecting mammals from the toxicity of ingested lipid hydroperoxides [43, 44]. Se dependent glutathione peroxidase is expressed at lower concentrations when the organism enters Se deficiency [45]. A possible explanation to our Se measurements in the proximal parts of the GI tract may therefore be a lower absorption of Se in proximal intestinal segments combined with lower expression of GSHPx-GI in MHD cases than controls. Less difference in Se levels between MHD cases and controls were found in caecum and colon. Significantly lower mean Se concentrations in skin ($P < 0.005$) and kidney ($P < 0.05$) samples from MHD cases were found, probably due to lowered selenoprotein expression during Se deficiency [46, 47].

No vitamin E analyses were included in this study due to budget limitations. Previous results have indicated that pigs with MHD have lower tissue alpha-tocopherol concentrations than the control pigs [26], and therefore its role in the pathogenesis of MHD needs further elucidation [2]. The trace elements calcium, copper, zinc, magnesium, iron would have been interesting to add to the analyses, since it has previously been described that Se deficiency can alter the distribution of other minerals [48, 49].

In other animals, like cattle, the Se requirements are differentiated according to muscularity [50]. The question whether lean, feed efficient pigs have higher Se requirements than slower growing pigs with a lower lean meat percentage is indeed worthwhile pursuing.

In conclusion, this study shows discrepancies in tissue Se concentrations between MHD cases and controls. As mentioned initially, the common practice regarding collection of samples from MHD cases in the field could be extended to include samples from the diaphragm or skeletal muscles for Se analysis, since these organ samples are easy accessible during field necropsy. Although the variation in Se concentrations of a given organ between MHD cases of different genetic origin and age was low, this report includes a limited number of pigs. Therefore our results should be affirmed by a larger randomized controlled trial.

Abbreviations

(ICP-MS): Inductively coupled plasma mass spectrometry; DM: Dry matter; GSHPx-GI: Intestinal form of glutathione peroxidase; HE: Hematoxylin and eosin; MHD: Mulberry Heart Disease; Q: Quadropoles; Se: Selenium; Te: Tellurium

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Authors' contributions

MOM, MF, BS, HW, AB and TF planned the study. MF, MOM and HW carried out necropsies and sample isolation. MV and BS were responsible for the selenium analyses. MOM drafted the manuscript. All authors read, commented on, and approved the final manuscript.

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Availability of data and materials

The data generated during the current case report are kept and stored by the corresponding author. The data are available from the corresponding author on reasonable request.

Ethics approval

No ethics approval was necessary since this case report describes diagnostic approaches and sampling procedures conducted by the veterinary pathologists during their search for the cause of sudden deaths in the pigs of the case herd.

Consent for publication

The farmers gave their consent to publish the results from the analyses of samples originating from their pigs.

Competing interests

The authors declare that they have no competing interests.

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References

1. Lingaas F, Brun E, Frøslie A. Estimates of heritability for selenium and glutathione peroxidase levels in pigs. J Anim Breed Genet. 1990;108:48–53.
2. Grant Maxie M, Robinson WF. In: Anim PD, editor. Mulberry heart disease of swine. 6th ed. London, UK: Elsevier; 2016. p. 39–41.
3. Loynachan AT. Mulberry heart disease. Dis swine. 10th ed. West Sussex, UK: Wiley-Blackwell Publishing Ltd; 2012. p. 192–3.
4. Falk M, Bernhoft A, Framstad T, Salbu B, Wisløff H, Kortner TM, et al. Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs. J Trace Elem Med Biol. 2018;50:527–36.
5. Rederstorff M, Krol A, Lescure A. Understanding the importance of selenium and selenoproteins in muscle function. Cell Mol Life Sci. 2006;63:52–9.
6. Whanger PD, Vendeland SC, Beilstein MA. Trace Elem Man Anim TEMA 8. Gersdorf, Germandy: Verlag Media Touristik; 1993. p. 119–26.

7. Jeong D, Kim TS, Chung YW, Lee BJ, Kim IY. Selenoprotein W is a glutathione-dependent antioxidant in vivo. FEBS Lett. 2002;517:225–8.
8. Yang JG, Hill KE, Burk RF. Dietary selenium intake controls rat plasma selenoprotein P concentration. J Nutr. 1989;119:1010–2.
9. Beckendorf L, Linke WA. Emerging importance of oxidative stress in regulating striated muscle elasticity. J Muscle Res Cell Motil. 2015;36:25–36.
10. Pallarés FJ, Yaeger MJ, Janke BH, Fernández G, Halbur PG. Vitamin E and selenium concentrations in livers of pigs diagnosed with mulberry heart disease. J Vet Diagn Investig Off Publ Am Assoc Vet Lab Diagn Inc. 2002;14:412–4.
11. Archibald AL, Bolund L, Churcher C, Fredholm M, Groenen MAM, Harlizius B, et al. Pig genome sequence--analysis and publication strategy. BMC Genomics. 2010;11:438.
12. Wijga S, Broekhuijse M, Gaustad AH, Duijvesteijn N, Grindflek E. Groundbreaking research for maximum genetic progress. Topigs Norsvin Annu Mag. 2016:58–61.
13. Cameron ND, Curran MK. Selection for components of efficient lean growth rate in pigs 4. Genetic and phenotypic parameter estimates and correlated responses in performance test traits with ad-libitum feeding. Anim Sci. 1994; 59:281–91.
14. Kerr JC, Cameron ND. Reproductive performance of pigs selected for components of efficient lean growth. Anim Sci. 1995;60:281–90.
15. Lonergan SM, Huff-Lonergan E, Rowe LJ, Kuhlers DL, Jungst SB. Selection for lean growth efficiency in Duroc pigs influences pork quality. J Anim Sci. 2001;79:2075–85.
16. Martinsen KH, Olsen D, Ødegård J, Meuwissen T. Economic values for lean meat and fat efficiency in Norwegian landrace nucleus pig population. Acta Agric Scand Sect — Anim Sci. 2016;66:145–51.
17. Patience JF, Rossoni-Serão MC, Gutiérrez NA. A review of feed efficiency in swine: biology and application. J Anim Sci Biotechnol. 2015;6:33.
18. Shen H, Thomas PR, Ensley SM, Kim W-I, Loynachan AT, Halbur PG, et al. Vitamin E and selenium levels are within normal range in pigs diagnosed with mulberry heart disease and evidence for viral involvement in the syndrome is lacking. Transbound Emerg Dis. 2011;58:483–91.
19. Nielsen TK, Wolstrup C, Schirmer AL, Jensen PT. Mulberry heart disease in young pigs without vitamin E and selenium deficiency. Vet Rec. 1989;124: 535–7.
20. Korpela H. Hepatic selenium concentration in pigs with microangiopathy (mulberry heart disease)--an animal model for the study of oxidative damage. Int J Vitam Nutr Res Int Z Vitam- Ernahrungsforschung J Int Vitaminol Nutr. 1990;60:156–8.
21. Sharp BA, Young LG, van DAA. Dietary induction of mulberry heart disease and Hepatosis Dietetica in pigs I. nutritional aspects. Can J Comp Med. 1972;36:371–6.
22. Brandt-Kjelsen A, Govasmark E, Haug A, Salbu B. Turnover of se in adequately fed chickens using se-75 as a tracer. J Anim Physiol Anim Nutr. 2014;98:547–58.
23. Moir DC, Masters HG. Hepatosis dietetica, nutritional myopathy, mulberry heart disease and associated hepatic selenium level in pigs. Aust Vet J. 1979;55:360–4.
24. Lindberg P, Siren M. Fluorometric selenium determination in the liver of normal pigs affected with nutritional muscular dystrophy and liver dystrophy. Acta Vet Scand. 6:59–64.
25. Liu J, Tuvblad C, Raine A, Baker L. Genetic and environmental influences on nutrient intake. Genes Nutr. 2013;8:241–52.
26. Rice DA, Kennedy S. Vitamin E, selenium, and polyunsaturated fatty acid concentrations and glutathione peroxidase activity in tissues from pigs with dietetic microangiopathy (mulberry heart disease). Am J Vet Res. 1989;50:2101–4.
27. Park S-Y, Gifford JR, Andtbacka RHI, Trinity JD, Hyngstrom JR, Garten RS, et al. Cardiac, skeletal, and smooth muscle mitochondrial respiration: are all mitochondria created equal? Am J Physiol - Heart Circ Physiol. 2014;307: H346–52.
28. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. J Cachexia Sarcopenia Muscle. 2017;8:349–69.
29. Weitzel F, Ursini F, Wendel A. Phospholipid hydroperoxide glutathione peroxidase in various mouse organs during selenium deficiency and repletion. Biochim Biophys Acta BBA - Gen Subj. 1990;1036:88–94.
30. Aragno M, Mastrocola R, Alloatti G, Vercellinatto I, Bardini P, Geuna S, et al. Oxidative stress triggers cardiac fibrosis in the heart of diabetic rats. Endocrinology. 2008;149:380–8.
31. Rocadembosch J, Amador J, Bernaus J, Font J, Fraile LJ. Production parameters and pig production cost: temporal evolution 2010–2014. Porc Health Manag [Internet. 2016;2 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5382395/.
32. Mauch E. The genetic basis of feed efficiency in swine divergently selected for residual feed intake. [Iowa, USA]: Iowa State University capstones; 2018.
33. Bereskin B, Hetzer HO. Genetic and maternal effects on pig weights, growth and probe backfat in diallel crosses of high- and low-fat lines of swine. J Anim Sci. 1986;63:395–408.
34. Gjerlaug-Enger E, Aass L, Odegård J, Vangen O. Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. Anim Int J Anim Biosci. 2010;4:1832–43.
35. Animalia, Norsvin. Ingris Årsstatistikk 2017 [Internet]. Norway; 2018. Report no.: 2018. Available from: https://www.animalia.no/contentassets/28e0db72674d496186f0570a9e606fca/arsstatistikk_2017.pdf
36. Animalia N. Ingris Årsstatistikk 2011 [Internet]. Norway; 2012. Report No.: 2011. Available from: https://www.animalia.no/contentassets/28e0db72674d496186f0570a9e606fca/arsstatistikk-2011.pdf
37. Beilharz RG, Luxford BG, Wilkinson JL. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? J Anim Breed Genet Z Tierzuchtung Zuchtungsbiologie. 1993;110:161–70.
38. van der Waaij EH. A resource allocation model describing consequences of artificial selection under metabolic stress. J Anim Sci. 2004;82:973–81.
39. Doeschl-Wilson AB, Kyriazakis I, Vincent A, Rothschild MF, Thacker E, Galina-Pantoja L. Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection. J Anim Sci. 2009;87:1638–47.
40. Rauw WM, Kanis E, Noordhuizen-Stassen EN, Grommers FJ. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livest Prod Sci. 1998;56:15–33.
41. Wright PL, Bell MC. Comparative metabolism of selenium and tellurium in sheep and swine. Am J Phys. 1966;211:6–10.
42. Suzuki H, Matsuzaki J, Hibi T. Ghrelin and oxidative stress in gastrointestinal tract. J Clin Biochem Nutr. 2011;48:122–5.
43. Chu FF, Esworthy RS. The expression of an intestinal form of glutathione peroxidase (GSHPx-GI) in rat intestinal epithelium. Arch Biochem Biophys. 1995;323:288–94.
44. Chu FF, Doroshow JH, Esworthy RS. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J Biol Chem. 1993;268:2571–6.
45. Burk RF. Molecular biology of selenium with implications for its metabolism. FASEB J Off Publ Fed Am Soc Exp Biol. 1991;5:2274–9.
46. Tsuji PA, Carlson BA, Anderson CB, Seifried HE, Hatfield DL, Howard MT. Dietary selenium levels affect Selenoprotein expression and support the interferon-γ and IL-6 immune response pathways in mice. Nutrients. 2015;7: 6529–49.
47. Malinouski M, Kehr S, Finney L, Vogt S, Carlson BA, Seravalli J, et al. High-resolution imaging of selenium in kidneys: a localized selenium Pool associated with glutathione peroxidase 3. Antioxid Redox Signal. 2012;16: 185–92.
48. Chareonpong-Kawamoto N, Yasumoto K. Selenium deficiency as a cause of overload of iron and unbalanced distribution of other minerals. Biosci Biotechnol Biochem. 1995;59:302–6.
49. Zhang Z, Liu M, Guan Z, Yang J, Liu Z, Xu S. Disbalance of calcium regulation-related genes in broiler hearts induced by selenium deficiency. Avian Pathol J WVPA. 2017;46:265–71.
50. Mehdi Y, Dufrasne I. Selenium in cattle: a review. Molecules. 2016;21.

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Research paper

Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs

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ABSTRACT

The study was conducted to compare effects of different dietary Se sources (sodium selenite [NaSe], Se-enriched yeast [Se yeast] or L-selenomethionine [SeMet]) and one Se-deficient control diet on the expression of selected genes, hematological and clinical biochemical parameters, and muscle morphology in two parallel trials with finisher pigs. Se concentrations in blood plasma and tissues were also monitored. From the pigs in one of the parallel groups, muscle samples obtained from *Musculus longissimus dorsi* (LD) before and during the trial were examined. The pigs in the other parallel group were challenged once with lipopolysaccharide (LPS) intravenously.

Transcriptional analyses of LD showed that selenogenes *SelenoW* and *H* were higher expressed in pigs fed Se-supplemented diets compared with control. Furthermore, the expression of interferon gamma and cyclooxygenase 2 was lower in the Se-supplemented pigs versus control. In whole blood samples prior to LPS, *SelenoN, SelenoS* and *thioredoxin reductase 1* were higher expressed in pigs fed NaSe supplemented feed compared with the other groups, possibly indicating a higher level of oxidative stress. After LPS exposure *glutathione peroxidase 1* and *SelenoN* were more reduced in pigs fed NaSe compared with pigs fed organic Se. Products of most above-mentioned genes are intertwined with the oxidant-antioxidant system. No significant effects of Se-source were found on hematologic parameters or microscopic anatomy. The Se-concentrations in various skeletal muscles and heart muscle were significantly different between the groups, with highest concentrations in pigs fed SeMet, followed by those fed Se yeast, NaSe, and control diet.

Consistent with previous reports our results indicate that dietary Se at adequate levels can support the body's antioxidant system. Our results indicate that muscle fibers of pigs fed organic Se are less vulnerable to oxidative stress compared with the other groups.

1. Introduction

Mulberry Heart Disease (MHD) and nutritional myopathy (skeletal muscle degeneration, NMD) are challenges in Norwegian pig production [1]. They are linked to selenium (Se)/vitamin E (vitE) deficiency [2]. Commercial feed mills fortify diets with the maximum allowable quantity of Se combined with high dietary vitE levels, but MHD and NMD still occur [1]. In intensive pig production daily weight gain and feed efficiency are high and infectious disease incidence is low [3–5]. However, high growth rate is associated with enhanced levels of oxidative stress [6,7] and is possibly linked to higher prevalence of MHD [8]. Se-/vitE-levels previously considered satisfactory are reported in pigs with pathognomonic MHD changes [8–11]. This raises the question whether existing feeding strategies meet Se requirements in high-yielding pigs [12,13]. Tissue Se concentrations and some selenoproteins mirror the Se status [14–16]. Biomarkers for detection of subclinical Se deficiency could possibly contribute to reveal MHD and NMD cases at an early stage.

Borella et al. [17] suggested that biological effects of Se in mammals are strongly influenced by the chemical Se form absorbed. Se source

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and status may also influence other body mineral elements. Molybdenum (Mo) is involved in the Se metabolism as an enzymatic cofactor [18,19], whereas magnesium (Mg) shows antioxidant properties and possibly contributes to the protection against MHD via its involvement in calcium homeostasis and glutathione synthesis [20–22].

The objectives of this study were to compare effects of three Se supplemented diets with a low Se control diet on: 1) immune and inflammatory responses in blood and muscle, 2) hematology and clinical biochemistry, 3) plasma mineral concentrations, 4) muscle histopathology and 5) tissue Se deposition. In addition, we wanted to identify biomarkers reflecting porcine Se status.

2. Material and methods

2.1. Experimental design and sampling

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

Twenty-four female finisher pigs, Landrace × Yorkshire × Duroc (LYDD) hybrids, with an average body weight of 25.6 (SD 4.4) kg were included in this study. The pigs were randomly allocated to four groups with two replicates in two rooms (room A and B), where they were kept in groups of three animals/pen. The pen size was 3.5 m^2 in room A and 2.2 m^2 in room B. Pigs in room A were kept until reaching slaughter weight (day 64 of trial period), thus requiring more space. Those in room B were killed when reaching 70 (SD 9.34) kg (see *LPS treatment*). The room temperature was initially set to 22 °C and successively reduced to 19 °C over the trial period. Dried compound feed was provided through a feed automate (Domino, type FR-H3 45L, Felleskjøpet, Norway). Feed and water were given *ad libitum*. Before entering the study, the pigs were fed standard compound starter feed containing 18% crude protein, 1.2% lysine, 5.3% crude fat, 8000 IU vitamin A, 1500 IU vitamin D, 150 mg vitE/kg, 12 mg copper/kg (from copper sulphate), 0.4 mg Se/kg (from NaSe) and 141 mg zinc/kg. After an acclimatization period of one week, the pigs were introduced to the trial diets. The composition of the trial feed is listed in Table 1. The trial diets were either the Se deficient, non-supplemented basal diet containing 0.05 mg Se/kg (control) or the same diet supplemented with NaSe (Mikromin Selen 300 FK,Vilomix, Norway), selenized yeast (Se yeast, Sel-Plex®, Alltech, USA) or L-selenomethionine (SeMet, Excential Selenium 4000 ®, Orffa, Netherlands). The NaSe-, Se yeast- and SeMet-enriched diets contained 0.33, 0.32 and 0.32 mg Se/kg, respectively. All diets were added 100 mg vitE/kg feed. The pigs in room A were fed with the trial feed to for 64 days, and were then slaughtered by standard procedures at the abattoir (CO_2). The pigs in room B received the trial feed for 47–48 days before they were euthanized by captive bolt stunning and exsanguination. These pigs were necropsied.

2.1.1. Muscle biopsies

Two muscle biopsies were obtained from the pigs in room A, the first one before starting the trial and the second biopsy on day 38. The 12 pigs were sedated using 2 mg azaperone/kg BW and locally anesthetized with lidocaine-adrenaline (20 mg/mL + 0.036 mg/mL). Biopsies were obtained by using a biopsy gun (Biopty-Cut®, BARD, USA) and disposable needles (14 g × 10 cm length, Biopty-Cut®, BARD, USA). Biopsy samples of approximately 25 mg and 75 mg were fixed in RNAlater for subsequent RNA isolation or frozen at −20 °C for total Se measurements by inductively coupled plasma mass spectrometry (ICP-MS), respectively. After the biopsy procedure, pigs received an i.m. injection of 0.4 mg meloxicam/kg BW.

2.1.2. Post mortem collection of tissue samples

Samples of selected skeletal muscles (LD, *M. semitendinosus*, *M. semimembranosus*, *M. biceps brachii*, *M. psoas major*, *M. quadriceps femoris*, diaphragm), heart muscle (left and right ventricle, septum), liver and kidney (cortex) were collected *post mortem*. Tissue samples of approximately 15 × 10 × 5 mm were fixed in 4% neutral buffered formaldehyde and processed routinely for light microscopic examination. Sections were cut at 5 µm and stained with hematoxylin and eosin (HE). For ICP-MS- analysis tissue samples were stored at −20 °C until analyses. For subsequent gene expression studies samples from LD were fixed in RNA-later for 24 h at 4 °C and subsequently stored at -20 °C until analysis.

Table 1
Composition of the trial diets.

Raw materials	%
Barley	59.2
Extracted soy	13.4
Wheat	10.0
Rapeseed cake Mestilla	5.00
Oats	5.00
Soybean oil (raw)	3.24
Limestone, Visnes	1.32
Mono Calcium Phosphate	0.45
NaCl	0.58
Mikromin Pig[a]	0.16
Vitamine A	0.05
VitE (V5)	0.06
Vitamin ADKB[b]	0.06
L-lysine	0.37
DL-metionine	0.11
L-treonine	0.17
L-tryptophan	0.01
Formic acid 85%	0.75
Physyme XP 5000 TPT	0.01
Total Selenium in mg Se/kg diet[c]	
None (control)	0.05
Sodium selenite (NaSe)	0.33
Se yeast (Se yeast)	0.32
L-SeMet	0.32

[a,b]Supplied the following per kilogram of diet.
[a] Fe 96 mg; Cu 20.8 mg; Mn 48 mg; Zn 96 mg; I 0.48 mg.
[b] Vitamin A 5700 IU; Vitamin D 1200 IU; Vitamin E 100 mg; Vitamin K 3.72 mg; Vitamin B1 2.4 mg; Vitamin B2 4.5 mg; Vitamin B5 12.0 mg; Vitamin B6 7.2 mg; Vitamin B12 0.012 mg; Folic acid 1.8 mg; Biotin 0.24 mg.
[c] as measured with ICP-MS.

2.1.3. Blood samples

Nine mL PAXgene®-tubes, Vacuette® Z serum clot activator and six mL Vacuette® Lithium Heparin tubes (Greiner Bio-One, Austria) along with Venoject needles (20G × 1½"UTW, USA) were used for blood sampling from the *Vena jugularis externa*. PAXgene®-tubes were frozen at -20 °C after two hours at room temperature and stored at -70 °C until RNA isolation. Blood samples for serum and plasma isolation were centrifuged at 3500 × *g* for 15 min (Megafuge 1.0 R, Heraeus SEPATECH, USA). Initial blood samples were obtained from pigs in both rooms after the acclimatization period. Subsequent blood samples were drawn from the pigs in room B prior to LPS-application (initiation LPS), one hour and 24 h after LPS treatment.

2.1.4. LPS treatment

Forty-five to forty-seven days into the trial, the twelve pigs in room B, with an average BW of 70 (SD 9.34) kg, received an injection of 2 µg LPS/kg BW (*E. coli* O111:B4, L4391, lot: 014M4019 V, G-irradiated, Sigma Aldrich, USA). Clinical examinations including rectal temperature, respiratory and heart rate were conducted before each blood sampling and at 90 min, 3 and 5 and 8 h after LPS application.

2.2. RNA isolation and quantitative Real Time PCR (qPCR) analysis

Total RNA was extracted in a randomized order. Blood RNA was isolated using the PAXgene®-products and the appendant protocol (PreAnalytiX®, UK). Muscle RNA was isolated using RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's protocol. RNA integrity was verified by the 2100 Bioanalyzer in combination with an RNA Nano Chip (Agilent Technologies), and RNA purity and concentrations were measured using the NanoDrop ND-1000 (NanoDrop Technologies). Samples with RNA integrity number (RIN) >7.5 were accepted for gene expression analysis. Total RNA was stored at −80 °C until use.

The qPCR assays were performed according to MIQE standards [23] on three animals from each of the four diet groups (Table 1) and at three time points. First-strand cDNA was synthesized from 0.8 µg total RNA from all samples using SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen™) following manufacturer's protocol. Negative controls were performed in parallel by omitting RNA or enzyme. Obtained cDNA was diluted 1:10 in molecular grade H_2O and stored at − 20 °C. The qPCR primers were obtained from the literature or designed using Primer3 (http://bioinfo.ut.ee/primer3/). Primer details are shown in Table A1 (supplementary material). All primer pairs gave a single band pattern for the expected amplicon of interest in all reactions. PCR reaction efficiency for each gene assay was determined using 2-fold serial dilutions of randomly pooled cDNA. The qPCR assays were performed using a LightCycler® 96 Real-Time PCR System (Roche Diagnostics). Each 10 µL DNA amplification reaction contained 2 µL PCR-grade water, 2 µL of 1:10 diluted cDNA template, 5 µL of Lightcycler 480 SYBR Green I Master (Roche Diagnostics) and 0.5 µL (final concentration 500 nM) of each forward and reverse primer. Each sample was assayed in duplicate, including a no template control (NTC). The three-step qPCR program included an enzyme activation step at 95 °C (5 min) and a minimum of 40 cycles of 95 °C (10 s), primer specific annealing temperature (10 s) and 72 °C (15 s). To confirm amplification specificity the PCR products from each primer pair were subjected to melting curve analysis and visual inspection of PCR products after each run by agarose gel electrophoresis. For target gene normalization, *Rpl4, Hprt1, B2m, Actb* and *Gapdh* were evaluated for use as reference genes by ranking relative gene expression according to their overall coefficient of variation (CV) and their interspecific variance, as described previously [24]. For muscle samples, *Hprt1* was used as a normalization factor, whereas the geometric average of *Hprt1*, *Rpl4* and *Gapdh* was used for blood samples. Mean normalized levels (MNE) of target genes were calculated from raw quantification cycle (Cq) values [25]. The panel of genes (font: *italic*) included in the study encode for different selenoproteins (font: regular), immune and inflammation related proteins (font: regular): a.) glutathione peroxidase 1 and 3 (*Gpx1, Gpx3*), iodothyronine deiodinases 1 and 3 (*Dio1*, *Dio3*) selenoproteins (Sel) H, K, S, W1, P1 and N1 (*SelenoH, SelenoK, SelenoS, SelenoW, SelenoP, SelenoN*) and thioredoxin reductase 1 (*Txnrd1*), b.) interleukin 1 β, 6 and 10 (*Il1β, Il6, Il10*), tumor necrosis factor alpha (*Tnfα*) and interferon gamma (*Ifnγ*), c.) superoxide dismutase 1 (*Sod1*), catalase (*Cat*), inducible nitric oxide synthase (*iNos*), cluster of differentiation 4 (*CD4*) and cyclooxygenase-2 (*Cox2*, syn. inducible prostaglandin endoperoxide H synthase) were measured in blood prior to and after LPS-challenge as well as in loin muscle biopsies (LD) collected repeatedly. Additionally, the gene expression of F-Box protein 32 (*Fbxo32*) and caspase 3 (*Casp3*) was analyzed in LD samples.

2.3. ICP-MS analysis

The concentrations of selected elements in tissue (dry matter, DM) and plasma samples were measured by ICP-MS (Agilent 8800) [26]. The mineral panel included Se for all samples and additionally, concentrations of Mg, and Mo were measured in plasma. Plasma samples were thawed and sonicated for 10 min at room temperature (Ultrasonic cleaner, Biltema, Norway). Then, 0.5 mL were transferred to new plastic vials, weighed (Analytical Balance LC 620 P, Sartorius, Germany) and mixed with 4.4 mL of an alkaline diluent [10]. A tellurium (Te) internal standard was added to the samples to a final sample dilution of 1:9 [27], a modification of the method described by Gajek et al. [28].

2.4. Hematology and biochemistry

Blood samples were subjected to a complete multi parametric hematological analysis (ADVIA 2120 Hematology System, Siemens Healthcare GmbH). The hematological parameters analyzed were red blood cell count (RBC), haematocrit (Hct), haemoglobin (Hgb), white blood cell count (WBC), thrombocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The clinical biochemical parameters C-reactive protein (CRP, Randox, UK), γ-glutamyl transferase (GGT, Siemens Medical Solutions Diagnostics), glutamate dehydrogenase (GLDH, Randox, UK), creatine kinase (CK, Siemens Healthcare GmbH), and aspartate transaminase (AST, Siemens Healthcare GmbH) were analyzed on ADVIA 1800 (Siemens Healthcare GmbH)

2.5. Histopathological analysis

Samples from liver, myocardium (right and left ventricle, septum) and seven skeletal muscles, incl. diaphragm, were analyzed.

The histopathological findings in skeletal muscles were graded blindly on a semi-quantitative scale of severity (0–normal, 1–mild changes, 2–moderate changes, 3–severe changes). Mild changes were defined as scattered, swollen myocytes with loss of striation or a very low number of degenerating myocytes, sometimes with mild infiltration of macrophages. Moderate changes were defined as multifocal degenerating or necrotic myocytes with or without macrophage infiltration, and severe changes were defined as multifocal, relatively widespread degenerating or necrotic myocytes with or without macrophage infiltration. Findings in liver and heart were not graded.

2.6. Statistics

Levels of mRNA in samples from the skeletal muscle *M. longissimus dorsi* (LD) were measured at day 0, 38 and 64 days into the trial. Linear regression both with and without random effect was used to analyze the q-PCR values using the functions lm and lmer (package *lme4*) in RStudio [29]. *Diet* and *Time* were tested as factors describing the fixed effect and pig ID was tested as random effect. The effect of including/excluding variables and random effect was compared using Akaike's 'Information Criterion' (AIC). Prediction from the model selected was plotted to show the differences between diets over time, for each time point the differences between diets were marked with different letters.

The measured Se concentrations in LD were plotted using Excel 2013.

For qPCR-results from the LPS-study, we reported mean normalized values (MNE) as mean ± SEM (time point 0 h) and the percentaged change compared with prior to LPS for 1 h and 24 h (Table 2). MNE were obtained by determining the geometric mean of three of the reference genes used and normalizing the target genes to this geometric mean. The percentaged difference between prior to LPS and 1 and 24 h after LPS application, respectively, was calculated for all blood parameters, plasma mineral concentrations and qPCR-results as value after LPS minus value prior to LPS-application divided by value prior to LPS-application. Linear regression (lm in R) was used to compare initial values as well as the percentaged differences for each diet, to obtain eventual significant differences between the diets. The regressions were repeated such that all diets were used as control. Significant differences between diets are reported with different letters in Table 2.

Table 2
Gene expression in whole blood (MNE) and mineral concentrations in plasma (mg/kg) prior to LPS application and change after LPS-application compared with values at 0 h (%).

		Change over time after LPS (Mean ± SEM; %)	
	Initial MNE- levels (Mean ± SEM)	1 h	24 h
GENE EXPRESSION			
SelenoW			
Control	0.575 ± 0.049	−20 ± 14	−6 ± 11
NaSe	0.648 ± 0.096	−17 ± 9	−10 ± 6
Se yeast	0.810 ± 0.097	−19 ± 12	−19 ± 7
SeMet	0.780 ± 0.061	−27 ± 5	−10 ± 5
SelenoH			
Control	0.150 ± 0.016	−32 ± 7	−15 ± 3
NaSe	0.143 ± 0.008	−24 ± 5	−10 ± 6
Se yeast	0.150 ± 0.011	−13 ± 17	−8 ± 12
SeMet	0.150 ± 0.003	−28 ± 3	−1 ± 2
Gpx1			
Control	14.8 ± 1.35	111 ± 46	-22 ± 11^{ab}
NaSe	33.3 ± 11.8	44 ± 43	-45 ± 11^{a}
Se yeast	40.4 ± 5.60	51 ± 32	-4 ± 1^{b}
SeMet	29.4 ± 4.75	74 ± 27	3 ± 11^{b}
Gpx3			
Control	0.067 ± 0.062	313 ± 261	−2 ± 34
NaSe	0.528 ± 0.154	103 ± 76	−61 ± 11
Se yeast	0.296 ± 0.061	131 ± 72	−6 ± 6
SeMet	0.392 ± 0.267	206 ± 61	−13 ± 33
SelenoK			
Control	0.738 ± 0.060	79 ± 9	−24 ± 6
NaSe	0.751 ± 0.107	43 ± 15	−5 ± 23
Se yeast	0.666 ± 0.011	54 ± 23	−17 ± 9
SeMet	0.636 ± 0.092	90 ± 46	10 ± 12
SelenoN			
Control	0.014 ± 0.001^{a}	−75 ± 2	-26 ± 5^{ab}
NaSe	0.030 ± 0.001^{b}	−75 ± 3	-49 ± 4^{a}
Se yeast	0.021 ± 0.001^{c}	−68 ± 8	-25 ± 8^{ab}
SeMet	0.015 ± 0.001^{a}	−72 ± 1	-22 ± 6^{b}
SelenoP			
Control	0.098 ± 0.006	−18 ± 8	−20 ± 7
NaSe	0.099 ± 0.013	2 ± 24	−22 ± 17
Se yeast	0.113 ± 0.009	−15 ± 11	−33 ± 6
SeMet	0.112 ± 0.034	14 ± 32	−20 ± 20
SelenoS			
Control	0.173 ± 0.015^{a}	−44 ± 6	−16 ± 5
NaSe	0.230 ± 0.010^{b}	−45 ± 5	−28 ± 8
Se yeast	0.185 ± 0.007^{a}	−34 ± 12	−17 ± 10
SeMet	0.173 ± 0.008^{a}	−44 ± 6	−12 ± 4
Txnrd1			
Control	0.293 ± 0.024^{a}	-24 ± 4^{a}	16 ± 11
NaSe	0.355 ± 0.014^{b}	-1 ± 7^{b}	5 ± 12
Se yeast	0.296 ± 0.014^{a}	-1 ± 5^{b}	< 1 ± 3
SeMet	0.269 ± 0.013^{a}	-21 ± 3^{a}	14 ± 14
Cat			
Control	0.663 ± 0.039^{a}	12 ± 47	−9 ± 8
NaSe	1.295 ± 0.251^{b}	84 ± 29	−9 ± 23
Se yeast	0.566 ± 0.032^{a}	41 ± 21	5 ± 7
SeMet	0.617 ± 0.123^{a}	54 ± 13	12 ± 15
Il10			
Control	0.015 ± 0.002	48 ± 23	-18 ± 4^{a}
NaSe	0.021 ± 0.002	22 ± 36	-15 ± 7^{a}
Se yeast	0.017 ± 0.001	−17 ± 13	-31 ± 9^{a}
SeMet	0.008 ± 0.004	62 ± 22	46 ± 24^{b}
PLASMA-MINERAL CONCENTRATIONS			
Se			
Control	0.06 ± 0.01^{a}	−6 ± 3	−9 ± 2
NaSe	$0.14 \pm 0.00^{b*}$	−13 ± 3	−17 ± 5
Se yeast	$0.16 \pm 0.00^{b*}$	−8 ± 2	−10 ± 5
SeMet	$0.15 \pm 0.00^{b*}$	−9 ± 2	−13 ± 4
Mg			
Control	0.021 ± 0.00^{a}	19 ± 5	5 ± 1^{a}
NaSe	0.022 ± 0.00^{ab}	11 ± 2	15 ± 5^{b}
Se yeast	0.025 ± 0.00^{bc}	16 ± 7	0 ± 5^{c}
SeMet	0.026 ± 0.00^{c}	19 ± 3	0 ± 4^{c}

(*continued on next page*)

Table 2 (*continued*)

	Initial MNE- levels (Mean ± SEM)	Change over time after LPS (Mean ± SEM; %)	
		1 h	24 h
Mo			
Control	0.006 ± 0.00[a]	−1 ± 3[a]	−27 ± 6[ab]
NaSe	0.010 ± 0.00[b]	8 ± 1[ab]	−51 ± 7[b]
Se yeast	0.008 ± 0.00[ab]	11 ± 1[ab]	−35 ± 6[ab]
SeMet	0.007 ± 0.00[a]	12 ± 2[b]	−27 ± 2[a]

Genes are ordered in accordance to their sequence in Fig. 2. Data in column 1 beneath heading "Gene expression" are mean normalized expression (MNE) levels of n = 3 animals per diet in whole blood isolated prior to LPS challenge. Data shown in column 1 beneath heading "Plasma mineral concentrations" are plasma concentrations of selenium (Se), molybdenum (Mo) and magnesium (Mg) as measured prior to application of LPS (mg/kg). Data presented in columns 2 and 3 are percentaged changes over time after application of LPS compared with values obtained prior to LPS. The regressions were repeated such that all diets were used as control. Values in a column not connected by the same letter are significantly different ($P < 0.05$). *$P < 0.001$.
Control: 0.05 mg Se/kg diet; NaSe: sodium selenite 0.33 mg Se/kg diet; Se yeast: selenium yeast 0.32 mg Se/kg diet; SeMet: L-selenomethionine 0.32 mg Se/kg diet.

The significance level was set to $P < 0.05$. Deviant significance levels were indicated in the text.

3. Results

Prior to initiation of the feeding trial, the Se-concentrations (mg/kg) measured in LD (shown in Fig. 1) and in plasma (control 0.13 (0.02), NaSe 0.12 (0.01), Se yeast 0.13 (0.01), SeMet 0.11 (0.01)) were similar in the pigs in all four groups.

During the feeding trial, no clinical signs of Se deficiency were observed. Furthermore, no significant effects on complete blood count or clinical biochemical parameters related to diet were revealed (data not shown).

The i.v. application of LPS provoked hyperthermia, which peaked similarly in all four groups after about 1 ½ hours at a mean rectal temperature of 41.2 (0.3) °C. Additionally, the pigs showed typical clinical signs associated with pyrexia including anorexia, lethargy and somnolence for up to 5 h after LPS application (data not shown) [30].

3.1. Gene expression patterns in M. longissimus dorsi

Fig. 2 shows expression levels of selected genes in LD samples. The selenogenes *SelenoW* and *SelenoH* showed similar expression patterns on days 38 and 64. These genes were higher expressed in all groups fed Se supplemented feed compared with the control group, but *SelenoH* in pigs fed SeMet was not statistically significantly different from control at day 38.

The expression of *Gpx3* was lower in pigs receiving Se supplemented feed (trend Se yeast: P = 0.07) at day 38 compared with control. However, at day 64 *Gpx3* expression was equal or significantly increased (SeMet) compared with control. The different Se treatments did not influence the expression of the selenogenes *SelenoK*, *SelenoN*, *SelenoP*, *SelenoS, Txnrd1* and *Dio1* and *3*. Data regarding *Dio1* and *3* are not shown. The expression levels of *Cox2*, the gene encoding the key inflammatory enzyme Cox2, were lower in pigs fed organic Se (Se yeast only at 38 days) compared with those fed NaSe and the control diet. In addition, the gene encoding the key apoptotic protease Casp3 was significantly lower expressed in pigs fed organic Se compared with pigs fed NaSe and the control diet at day 38 but not at day 64.

The expression of the gene *Sod1*, encoding the copper and zinc-dependent key antioxidant enzyme Sod1, was lower in pigs fed the Se supplemented diets compared with control at day 38. On day 64, there was no difference between groups. For the gene encoding the cytokine Ifnγ, no difference between feed groups were found at day 38, but lower expression in the Se-supplemented pigs, particularly in those fed organic Se, was found at day 64.

The expression of the non-selenogenes *Cat*, *CD4, Fbxo32, Il1β*, *Il6*, *Il10* and *iNos* was not influenced by Se treatment (only *Cat*-related data shown in Fig. 2).

3.2. Gene expression patterns and mineral-concentrations in blood prior to LPS injection

SelenoN, *SelenoS* and *Txnrd1*, as well as the gene *Cat*, encoding a key antioxidant enzyme, were highest expressed in the pigs fed NaSe compared with the other groups (Table 2). However, for the selenogenes *SelenoW, SelenoH*, *Gpx1* and *3*, *SelenoK*, *SelenoP, Dio1* and *3* as well as for the non-selenogenes *Cox2*, *Sod1*, *Ifnγ*, *Il1β*, *Il6*, *Il10*, *CD4* and *iNos* no differences between groups were found. Data for *Dio1* and *3*, *CD4*, *Cox2*, *Sod1*, *Il1β, Il6*, *Ifnγ*, and *iNos* are not shown.

Pigs receiving Se-supplemented diets showed significant higher plasma-Se compared with the control ($P < 0.001$), but no difference in plasma-Se was found between pigs fed the various Se supplemented diets (Table 2).

The plasma concentration of Mg was higher in the pigs fed organic Se (Se yeast and SeMet) compared with the control and pigs fed NaSe (Table 2). The plasma concentration of Mo was higher in the pigs fed the NaSe supplemented diet compared with the control and pigs fed SeMet (Table 2).

3.3. Gene expression profiles and mineral concentrations in blood after LPS challenge

Without significant differences between groups, the selenogenes *Gpx1*, *Gpx3* and *SelenoK* as well as the non-selenogene *Il1β* were up-regulated within 1 h after LPS challenge, whereas *SelenoN*, *SelenoS*, *SelenoH* and *SelenoW* were down-regulated. Most of these genes had returned to the baseline levels after 24 h. For *SelenoP* and *SelenoS* lower levels than initial expression levels were found after 24 h.

While unchanged in pigs supplemented with NaSe and Se yeast, the expression of *Txnrd1* was down-regulated from the initiation to 1 h after LPS-injection in control and SeMet fed pigs (Table 2).

Twenty-four hours after LPS-injection (Table 2), in pigs fed NaSe, *Gpx1* was more reduced compared with pigs fed SeMet, and *SelenoN* compared with SeMet and Se yeast. The *Il10*-gene expression was down-regulated in the control, NaSe and Se yeast compared with SeMet which was up-regulated. Diet nor LPS did not influence the expression of *Dio1* and *3*, *CD4*, *Il6* and *iNos* (data not shown).

The plasma-Mo-concentrations reached higher concentrations in the pigs supplemented with SeMet compared with the control group at 1 h after LPS. A following decrease in plasma-Mo was strongest in NaSe supplemented pigs compared with SeMet. The plasma-Mg-levels

increased more in control and NaSe-group compared with the groups supplemented with Se yeast and SeMet at 24 h.

3.4. Selenium concentrations in tissue samples

Se concentrations in LD biopsies showed a pronounced increase during the trial period in pigs fed organic Se (SeMet or Se yeast) supplemented feed compared with pigs fed dietary NaSe and control ($P < 0.001$, Fig. 1). Furthermore, at the end of the trial, all skeletal muscle and heart muscle samples from pigs fed organic dietary Se sources showed increased Se concentrations compared with those fed NaSe or no Se supplement (Table 3); SeMet > Se yeast > NaSe > unsupplemented diets. Pigs fed Se supplemented feed also showed higher Se concentrations in liver samples compared with control. However, Se-concentrations in liver and kidney of all Se supplemented pigs were more similar than those in muscle samples.

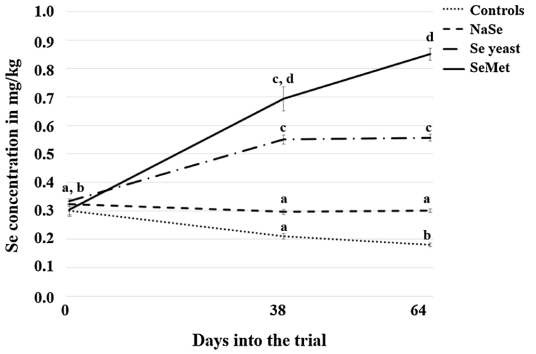

Fig. 1. Effect of time and diet on Se-concentration in *M. longissimus dorsi* (LD, mg/kg DM, Mean ± SEM).
Se-concentrations in LD during the trial period of 64 days (mean ± SEM). Different letters denote significant differences in Se concentration in LD between groups (control: 0.05 mg Se/kg diet; NaSe: sodium selenite 0.33 mg Se/kg diet; Se yeast: selenium yeast 0.32 mg Se/kg diet; SeMet: L-selenomethionine 0.32 mg Se/kg diet) and time point ($P < 0.05$ for the linear model (R lm)).

3.5. Histopathology

The two muscles most commonly showing degenerative changes were *M. semitendinosus* and LD. In all groups, these two muscles showed mild to moderate degenerative changes in most individuals. In the myocardium (heart muscle), scattered myocytes with vacuolization of the cytoplasm were observed in several pigs without significant differences between groups. Within all four groups, multifocal hepatic hemorrhages were more often observed in LPS-treated animals than in slaughtered animals.

Unfortunately, the low number of animals per group in this study potentially increased the influence of inter-individual variance. This might have reduced the possibilities to find significant differences in clinical observations, haematology, biochemistry, and histopathology as well as for the expression of some seleno- and non-selenogenes and presented mineral concentrations.

4. Discussion

Diet significantly influenced gene expression of some seleno- and non-selenogenes in both LD and whole blood obtained prior to and after application of LPS. Furthermore, we observed effects of diet and LPS on plasma-Mo and Mg in addition to the influence on plasma- and tissue Se concentrations.

4.1. Gene expression in M. longissimus dorsi

The expression of *SelenoW* and *SelenoH* in LD samples was found to depend on dietary Se, which is in line with other studies [31–35]. The marked reduction of the expression of *SelenoW* in the control pigs might have been a result of biological readjustment to stabilize the Se levels in brain and endocrine organs at the expense of tissues like skeletal muscle in cases of limited Se supply [36]. Resistance to oxidative stress conferred by SelW depended on GSH [37]. SelH was shown to regulate redox homeostasis and to suppress DNA damage [38]. In addition, SelH regulated expression levels of genes involved in *de novo* GSH synthesis [38]. Gene expression of *SelenoW* and *SelenoH* might be coupled via the GSH metabolism. Other authors found a positive correlation between *SelenoW* mRNA levels and intracellular GSH [39–41], which could also explain the above mentioned downregulation of *SelenoW* in the control group. The stronger upregulation of *SelenoH* in NaSe fed pigs compared with those fed Se yeast at day 64 might relate to the reactive oxygen species (ROS) generation during the NaSe metabolism [42–47]. The reason for the concurrent different expression of *SelenoH* in Se yeast and SeMet fed pigs remained unknown to the authors.

In LD biopsies from day 38, both *Gpx3* and *Sod1* were higher expressed in control compared with pigs fed Se-supplemented diets. This suggests increased O_2^- dismutation (Sod1) followed by upregulated decomposition of a possible product, hydrogen peroxide (H_2O_2), catalyzed by Gpx3. It is possible that higher levels of ROS upregulated both *Cox2* and *Casp3* expression in pigs fed NaSe and in control pigs, compared with those fed SeMet supplemented feed. Low Se status has been shown to aggravate the Casp3-dependent apoptotic response, probably due to impaired capacity of Gpx1 to degrade H_2O_2 [48]. On the other hand, NaSe induced ROS production in combination with growth-dependent ROS production [6,7], might have exhausted this capacity of Gpx1. Higher *Gpx3*-expression levels in LD from SeMet fed pigs at day 64, compared with control, potentially led to stronger cellular protection against oxidative stress as described by Stiegler et al. [49]. In contrast to the biopsies, the last LD samples were isolated after commercial slaughter. Ex-sanguination increases the level of oxidative stress and could explain the observed upregulation of the genes encoding the antioxidative selenoproteins Gpx3 and SelH in the Se-supplemented groups. The missing upregulation of *Gpx3* and *SelenoH* in the control pigs might result from the hierarchy of selenoproteins, since the two corresponding proteins are not prioritized in case of low Se supply [50,51]. The reason for the different expression of *Gpx1* (day 38) in the LD of pigs fed Se yeast versus those fed SeMet remained unknown to the authors but might relate to the antioxidative effects of other Se compounds found in Se yeast [41]. Looking at the gene expression in samples isolated prior to the feeding trial, the same patterns for *SelenoH* and *GPx1* could be seen. Thus, something extraneous to our trial might have had influence.

The lower expression of *Ifn*γ in the Se-supplemented pigs compared with control might also relate to ROS, which have been shown to enhance the *Ifn*γ expression [52]. Dependent on time and diet, the expression of *Cox2* in LD samples increased throughout the study period, but more in control pigs and those fed the NaSe supplemented diet. Low Se status and elevated levels of ROS have been shown to upregulate the expression of *Cox2* [53–55]. In summary, our observations might mirror a higher antioxidant capacity in skeletal muscle from pigs supplemented with organic Se.

The expression of several selenogenes in the control pigs was down-

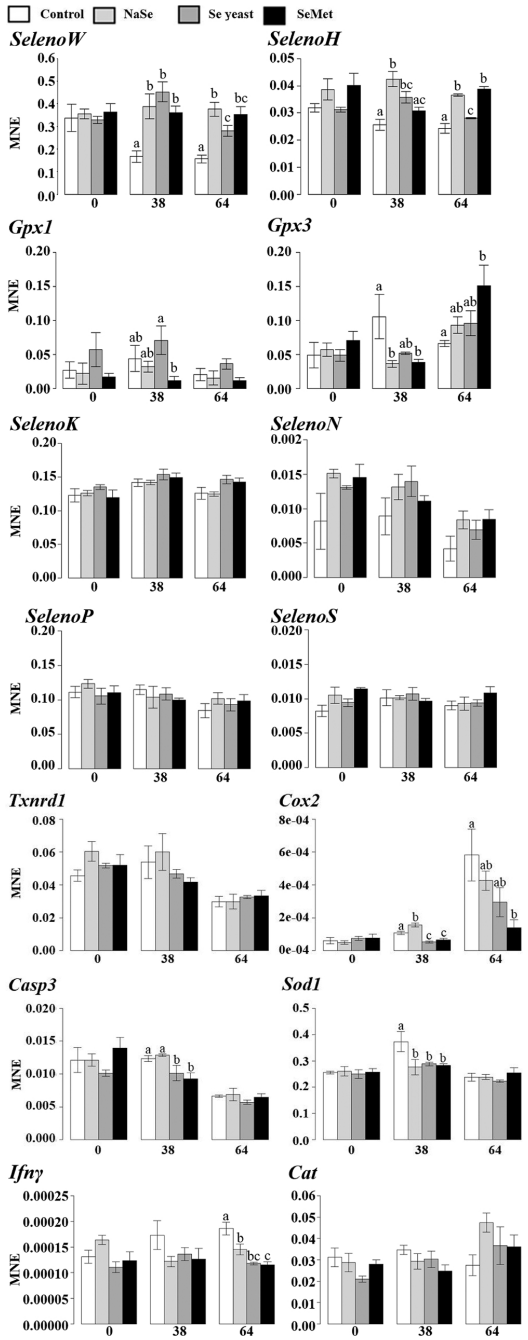

Fig. 2. Gene expression in *M. longissimus dorsi* of selected genes included in the study (MNE, Mean ± SEM).
Data are mean normalized levels of expression (MNE) of n = 3 animals per diet group and sampling time point in *M. longissimus dorsi*. Mean levels are shown in barplots with standard error of the mean (SEM). Different letters denote significant differences in expression levels between groups (control: 0.05 mg Se/kg diet; NaSe: sodium selenite 0.33 mg Se/kg diet; Se yeast: selenium yeast 0.32 mg Se/kg diet; SeMet: L-selenomethionine 0.32 mg Se/kg diet) at each time point. Different letters indicate $P < 0.05$ for the linear model (R lm).

regulated. The parallel-observed higher expression of some genes encoding important Se independent antioxidants might indicate compensatory up-regulation in the control pigs.

4.2. Gene expression in whole blood before and after LPS injection

Prior to LPS injection, *SelenoN*, *SelenoS* and *Txnrd1* as well as *Cat* were higher expressed in the blood of pigs fed NaSe. NaSe increases the Txnrd1 activity [56] as this selenoenzyme is implicated in the reductive assimilation of NaSe by generating selenide for selenoprotein synthesis [57]. Metabolizing the rather strong oxidizing NaSe can trigger endoplasmic reticulum (ER) stress due to generation of ROS [42–47]. Both SelS, an ER stress response protein [58], and SelN1 protect the ER from ROS [59,60]. *Cat* was higher expressed in whole blood of pigs fed NaSe. The antioxidant enzyme Cat, found in all aerobic organisms, catalyzes the decomposition of the ROS H_2O_2 to oxygen and water [61]. As mentioned above, NaSe metabolism can increase the ROS production, which in turn may have increased the requirement for the enzyme Cat to prevent oxidative damage.

The LPS dose of 2 µg/kg BW has previously been shown to induce the aforementioned clinical signs in pigs [62]. In accordance with others, our results clearly showed significant influence of LPS on gene expression [63]. LPS triggered oxidative stress may have caused the upregulation of *Gpx1* and *Gpx3* during the acute phase [64,65]. The increased *SelenoK* mRNA-levels 1 h after LPS challenge coincided with a drop in WBC-counts (data not shown) which can be explained by transendothelial migration of leucocytes. SelK is important for Ca^{2+}-dependent functions in leucocytes, which include transendothelial migration [66–68] explaining its early upregulation. The parallel downregulation of *SelenoN* could be linked to the functional relationship of SelN1 with the ER Ca^{2+} import SERCA2 pump [69]. SelN1 enhances SERCA2 activity [69], which would counteract the aforementioned function of SelK. A distinct SelN1 to SelK ratio might be the prerequisite for transendothelial migration of leucocytes. The enhanced downregulation of *SelenoN* in NaSe fed pigs might be related to its function in regulation of the Ca^{2+} homeostasis in the cell [69] and possibly disturbs the SelN1 to SelK ratio. SelH protects intracellular GSH and antioxidant levels and increases the expression of key enzymes in GSH biosynthesis [38]. In mice, LPS mediated the inhibition of GSH synthesis [70] possibly explaining the observed downregulation of *SelenoH*-expression in blood samples from the pigs 1 h after LPS injection. Fast growing animals experience a higher level of oxidative stress as ROS-production is proportional to the respiratory activity of the cell [71,72] which may be added to NaSe- and LPS-related ROS in these pigs. The stronger downregulation of *Gpx1* in NaSe-fed pigs 24 h after LPS challenge compared with those fed organic Se could be due to re-direction of Se to more important selenoproteins [50,51,73].

In summary, these observations might reflect increased oxidative stress, in case of NaSe supply.

Table 3
Selenium concentration (mg/kg DM) in tissues collected post mortem after 64 days of trial duration.

Tissue	n	Control diet (0.05 mg Se/kg)	n	NaSe (0.33 mg Se/kg)	n	Se yeast (0.32 mg Se/kg)	n	SeMet (0.32 mg Se/kg)
Kidney	2	5.70 (0.28)	3	7.67 (0.84)	3	7.80 (0.95)	2	8.80 (1.56)
Liver	2	0.71 (0.01)^{a}	3	1.57 (0.06)^{b}	3	1.83 (0.12)^{c}	2	1.75 (0.07)^{c}
RV	3	0.34 (0.12)^{a}	3	0.78 (0.12)^{b}	3	1.05 (0.09)^{c}	3	1.20 (0.17)^{c}
LV	3	0.47 (0.01)^{a}	3	0.90 (0.05)^{b}	3	1.20 (0.00)^{c}	3	1.37 (0.06)^{d}
Septum	3	0.49 (0.04)^{a}	3	0.90 (0.01)^{b}	3	1.13 (0.06)^{c}	3	1.47 (0.06)^{d}
LD	3	0.18 (0.01)^{a}	3	0.30 (0.01)^{b}	3	0.56 (0.02)^{c}	3	0.85 (0.04)^{d}
MBB	3	0.21 (0.01)^{a}	3	0.40 (0.01)^{b}	3	0.63 (0.02)^{c}	3	0.96 (0.03)^{d}
MST	3	0.18 (0.03)^{a}	3	0.36 (0.02)^{b}	3	0.61 (0.02)^{c}	3	0.92 (0.01)^{d}
MQF	3	0.19 (0.01)^{a}	3	0.37 (0.01)^{b}	3	0.62 (0.03)^{c}	3	0.96 (0.01)^{d}
MPM	3	0.19 (0.01)^{a}	3	0.35 (0.02)^{b}	3	0.63 (0.02)^{c}	3	0.92 (0.03)^{d}
MSM	3	0.16 (0.02)^{a}	3	0.32 (0.01)^{b}	3	0.49 (0.04)^{c}	3	0.75 (0.10)^{d}
Diaphragm	2	0.21 (0.01)^{a}	3	0.46 (0.04)^{b}	3	0.62 (0.04)^{c}	3	0.95 (0.06)^{d}

M. longissimus dorsi (LD), *M. semitendinosus* (MST), *M. semimembranosus* (MSM), *M. biceps brachii* (MBB), *M. quadriceps femoris* (MQF), *M. psoas major* (MPM), diaphragm, myocardium (left and right ventricle [LV and RV], septum). Values are shown as Mean (SD). The regressions (R *lm*) were repeated such that all diets were used as control. Values in a row not connected by the same letter are significantly different ($P < 0.05$).

4.3. Se, Mo and Mg concentrations in plasma before and after LPS injection

Plasma Se concentrations increased significantly from the start of the study until day 46 (prior to LPS challenge) in pigs receiving diets supplemented with Se. A low plasma-Se-concentration, considered Se deficient [74], was shown for the control.

Although decreasing after LPS challenge in pigs fed Se supplemented diets, plasma-Se-concentrations did not reach levels considered Se deficient. In pigs, serum- or plasma-Se-concentrations between 0.005-0.06 mg/kg are classified as deficient [74]. In line with our study, other authors have shown a declining Se status during infections in humans, rodents and pigs [63,64,75–78], which might be due to a changed pattern of selenoprotein synthesis and endothelial binding of SelP1 [79–82]. The stable plasma-Se-concentrations observed during our LPS study in pigs receiving the control diet, however, did probably reflect reduced selenoprotein synthesis [83], since these animals already were low in Se when challenged with LPS.

The increase of plasma-Mo-concentrations shortly after LPS-application in the Se-supplemented groups might be related to the turnover of Se compounds and selenoproteins for liberation of Se via the transselenation pathway [73]. This support the above suggested lowered selenoprotein synthesis in control pigs. Mo-containing enzymes catalyze basic metabolic reactions in the sulfur (S) cycle [18]. As Se and S compounds have similar chemical/physical properties, they will largely follow the same metabolic routes [19].

The higher plasma-Mg-concentrations observed in pigs fed Se yeast and SeMet enriched diets versus pigs fed NaSe and control might relate to the antioxidant properties of Mg [21,22]. It counteracts the development of ROS-related diseases [84] and is essential for the synthesis of reduced GSH [85–88]. Sugimoto *et al.* showed that Mg decreases the cytokine production [89].

4.4. Se concentrations in tissues

Se concentrations in the porcine liver and kidney mirrored the Se-supplementation differently from all the muscles including the myocardium due to the relative high amount of SeCys in liver and kidney [90,91]. Incorporation of selenomethionine in non-selenoproteins led to higher muscle-Se-concentrations in the pigs fed diets supplemented with organic Se compared with both NaSe-fed and control pigs, being highest in SeMet pigs. In comparison to pure SeMet formulations Se yeast contains different Se compounds whereof selenomethionine accounted for 54–74% [92–94]. This might explain the significant different Se concentrations observed in the skeletal muscle samples from Se yeast compared with SeMet fed pigs.

In most of Scandinavia, some other parts of Europe and parts of China, local produced grain is low in Se [95]. Years after the replacement of imported Se-rich grain from the USA and Canada with more homegrown grains, the Norwegian population has showed a considerable decrease in serum-Se concentration [96]. Offering Se rich meat from animals fed organic Se sources, in particular selenomethionine, is one possibility to increase the Se status in Norwegian consumers.

4.5. Conclusions

SelenoW could be a good molecular marker of Se status as its expression was lower in skeletal muscle of the control animals fed a diet low in Se. NaSe at a dietary level of 0.3 mg Se/kg might have induced additional oxidative stress in the fast growing pig as indicated by the higher expression of several seleno- and non- selenogenes compared with control, SeMet and Se yeast. This, together with the higher plasma Mg concentrations in pigs fed organic Se, possibly reflect that organic Se positively influenced the pigs' immune response and antioxidant capacity. Future studies might focus on the impact of non-toxic levels of NaSe, Se yeast and SeMet on antioxidative active compounds like e.g. GSH or thiol groups. In addition, more knowledge is crucial to understand the relations between selenoproteins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2018.03.003.

References

[1] Norwegian Meat and Poultry Research Center, Helsetjenesten for svin – Årsrapport 2011 [Pig Health Services – Annual report 2011], http://www.animalia.no/upload/FIler%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport2011.pdf, 2012.

[2] S. Done, S.M. Williamson, B.W. Strugnell, Nervous and locomotor systems. in: J.J. Zimmerman, L.A. Karriker, A. Ramirez, K.J. Schwartz, G.W. Stevenson (Eds.), Diseases of Swine, 10th edition, John Wiley and Sons Ltd., UK, 2012, pp. 294–328.

[3] Norwegian Meat and Poultry Research Center, Kjøttets tilstand – Status i norsk kjøtt- og eggproduksjon [Report on the status of the Norwegian meat and egg production], http://flashbook.no/animalia/kjottetstilstand16/pubData/source/161684-Animalia-KT16-13-DSA.pdf, 2017.

[4] Norwegian Meat and Poultry Research Center, Helsetjenesten for svin – Årsrapport 2015 [Pig Health Services – Annual report 2015], http://www.animalia.no/upload/FIler%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport%202015.pdf, 2016.

[5] H.J. Jørgensen, B. Heier, K. Hauge, B.G. Herrador, M. Hofshagen, N.V. Institute (Ed.), The Norwegian Zoonoses Report 2015, Norwegian Veterinary Institute, 2016, p. 2016 http://www.vetinst.no/rapporter-og-publikasjoner/rapporter//norwegian-zoonoses-report-2015.

[6] C. Alonso-Alvarez, S. Bertrand, B. Faivre, G. Sorci, Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches, Funct. Ecol. 21 (5) (2007) 873–879.

[7] H.M. Brown-Borg, S.G. Rakoczy, Growth hormone administration to long-living dwarf mice alters multiple components of the antioxidative defense system, Mech. Ageing Dev. 124 (10-12) (2003) 1013–1024.

[8] T.K. Nielsen, C. Wolstrup, A.L. Schirmer, P.T. Jensen, Mulberry Heart Disease in young pigs without vitamin E and selenium deficiency, Vet. Rec. 124 (20) (1989) 535–537.

[9] H. Shen, P.R. Thomas, S.M. Ensley, W.I. Kim, A.T. Loynachan, P.G. Halbur, T. Opriessnig, Vitamin E and selenium levels are within normal range in pigs diagnosed with Mulberry Heart Disease and evidence for viral involvement in the syndrome is lacking, Transbound. Emerg. Dis. 58 (6) (2011) 483–491.

[10] F.J. Pallarés, M.J. Yaeger, B.H. Janke, G. Fernandez, P.G. Halbur, Vitamin E and selenium concentrations in livers of pigs diagnosed with mulberry heart disease, J. Vet. Diagn. Invest. 14 (5) (2002) 412–414.

[11] D.A. Rice, S. Kennedy, Vitamin E selenium, and polyunsaturated fatty acid concentrations and glutathione peroxidase activity in tissues from pigs with dietetic microangiopathy (mulberry heart disease), Am. J. Vet. Res. 50 (12) (1989) 2101–2104.

[12] T. Sivertsen, E. Vie, A. Bernhoft, B. Baustad, Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds, Acta Vet. Scand. 49 (2007) 1.

[13] M. Oropeza-Moe, H. Wisloff, A. Bernhoft, Selenium deficiency associated porcine and human cardiomyopathies, J. Trace Elem. Med. Biol. 31 (2015) 148–156.

[14] J. Combs, F. Gerald, Biomarkers of selenium status, Nutrients 7 (4) (2015) 2209.

[15] A. Kipp, A. Banning, E.M. van Schothorst, C. Meplan, L. Schomburg, C. Evelo, S. Coort, S. Gaj, J. Keijer, J. Hesketh, R. Brigelius-Flohe, Four selenoproteins protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon, Mol. Nutr. Food Res. 53 (12) (2009) 1561–1572.

[16] A.P. Kipp, J. Frombach, S. Deubel, R. Brigelius-Flohe, Selenoprotein W as biomarker for the efficacy of selenium compounds to act as source for selenoprotein biosynthesis, Methods Enzymol. 527 (2013) 87–112.

[17] P. Borella, A. Bargellini, C.I. Medici, Chemical form of selenium greatly affects metal uptake and responses by cultured human lymphocytes, Biol. Trace Elem. Res. 51 (1) (1996) 43–54.

[18] C. Kisker, H. Schindelin, D.C. Rees, Molybdenum-cofactor-containing enzymes: structure and mechanism, Annu. Rev. Biochem. 66 (1997) 233–267.

[19] M. Birringer, S. Pilawa, L. Flohe, Trends in selenium biochemistry, Nat. Prod. Rep. 19 (6) (2002) 693–718.

[20] H. Korpela, Hypothesis: increased calcium and decreased magnesium in heart muscle and liver of pigs dying suddenly of microangiopathy (mulberry heart disease): an animal model for the study of oxidative damage, J. Am. Coll. Nutr. 10 (2) (1991) 127–131.

[21] D.M. El-Tanbouly, R.M. Abdelsalam, A.S. Attia, M.T. Abdel-Aziz, Pretreatment with magnesium ameliorates lipopolysaccharide-induced liver injury in mice, Pharmacol. Rep. 67 (5) (2015) 914–920.

[22] R.M. Touyz, Q. Pu, G. He, X. Chen, G. Yao, M.F. Neves, E. Viel, Effects of low dietary magnesium intake on development of hypertension in stroke-prone spontaneously hypertensive rats: role of reactive oxygen species, J. Hypertens. 20 (11) (2002) 2221–2232.

[23] S.A. Bustin, V. Benes, J.A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M.W. Pfaffl, G.L. Shipley, J. Vandesompele, C.T. Wittwer, The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments, Clin. Chem. 55 (4) (2009) 611–622.

[24] T.M. Kortner, E.C. Valen, H. Kortner, I.S. Marjara, A. Krogdahl, A.M. Bakke, Candidate reference genes for quantitative real-time PCR (qPCR) assays during development of a diet-related enteropathy in Atlantic salmon (Salmo salar L.) and the potential pitfalls of uncritical use of normalization software tools, Aquaculture 318 (3-4) (2011) 355–363.

[25] P.Y. Muller, H. Janovjak, A.R. Miserez, Z. Dobbie, Processing of gene expression data generated by quantitative real-time RT-PCR, Biotechniques 32 (6) (2002) 1372-4, 1376 1378-9.

[26] A. Brandt-Kjelsen, E. Govasmark, A. Haug, B. Salbu, Turnover of Se in adequately fed chickens using Se-75 as a tracer, J. Anim. Physiol. Anim. Nutr. (Berl) 98 (3) (2014) 547–558.

[27] A. Brandt-Kjelsen, Selenium from Feed to Fork, with Emphasis on Bioavailability and Bioaccessibility, Department of Environmental Sciences/CERAD CoE, Campus Ås, Norway, Norwegian University of Life Sciences, Norway, 2013, p. 73.

[28] R. Gajek, F. Barley, J. She, Determination of essential and toxic metals in blood by ICP-MS with calibration in synthetic matrix, Anal. Methods 5 (9) (2013) 2193–2202.

[29] RStudio Team, R Foundation for Statistical Computing (Ed.), RStudio Integrated Development for R, RStudio, Inc., Boston, USA, 2016.

[30] R.W. Johnson, E. von Borell, Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin, J. Anim. Sci. 72 (2) (1994) 309–314.

[31] B. Sun, R. Wang, J. Li, Z. Jiang, S. Xu, Dietary selenium affects selenoprotein W gene expression in the liver of chicken, Biol. Trace Elem. Res. 143 (3) (2011) 1516–1523.

[32] S.C. Vendeland, M.A. Beilstein, J.Y. Yeh, W. Ream, P.D. Whanger, Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium, Proc. Natl. Acad. Sci. U. S. A. 92 (19) (1995) 8749–8753.

[33] Q.P. Gu, W. Ream, P.D. Whanger, Selenoprotein W gene regulation by selenium in L8 cells, Biometals 15 (4) (2002) 411–420.

[34] R.A. Sunde, A.M. Raines, K.M. Barnes, J.K. Evenson, Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome, Biosci. Rep. 29 (5) (2009) 329–338.

[35] E.N. Terry, J.J. Michal, C.E. Hostetler, R.L. Kincaid, Levels of mRNA for three selenoproteins in skeletal muscle of fetal and newborn pigs, Livest. Sci. 124 (1-3) (2009) 21–25.

[36] D. Behne, H. Hilmert, S. Scheid, H. Gessner, W. Elger, Evidence for specific selenium target tissues and new biologically important selenoproteins, Biochim. Biophys. Acta 966 (1) (1988) 12–21.

[37] D.-w. Jeong, T.S. Kim, Y.W. Chung, B.J. Lee, I.Y. Kim, Selenoprotein W is a glutathione-dependent antioxidant in vivo, FEBS Lett. 517 (1–3) (2002) 225–228.

[38] J. Panee, Z.R. Stoytcheva, W. Liu, M.J. Berry, H. Selenoprotein, Is a redox-sensing high mobility group family DNA-binding protein that up-regulates genes involved in glutathione synthesis and phase II detoxification, J. Biol. Chem. 282 (33) (2007) 23759–23765.

[39] P.D. Whanger, Selenoprotein expression and function-selenoprotein W, Biochim. Biophys. Acta 1790 (11) (2009) 1448–1452.

[40] D.J. Hoffman, Role of selenium toxicity and oxidative stress in aquatic birds, Aquat. Toxicol. 57 (1) (2002) 11–26.

[41] M. Richie Jr, A. Das, A.M. Calcagnotto, R. Sinha, W. Neidig, J. Liao, E.J. Lengerich, A. Berg, T.J. Hartman, A. Ciccarella, A. Baker, M.G. Kaag, S. Goodin, R.S. DiPaola, K. El-Bayoumy, Comparative effects of two different forms of selenium on oxidative stress biomarkers in healthy men: a randomized clinical trial, Cancer Prev. Res. (Phila) 7 (8) (2014) 796–804.

[42] L. Guan, B. Han, Z. Li, F. Hua, F. Huang, W. Wei, Y. Yang, C. Xu, Sodium selenite induces apoptosis by ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction in human acute promyelocytic leukemia NB4 cells, Apoptosis 14 (2) (2009) 218–225.

[43] P. Palsamy, K.R. Bidasee, T. Shinohara, Selenite cataracts: activation of endoplasmic reticulum stress and loss of Nrf2/Keap1-dependent stress protection, Biochim. Biophys. Acta 1842 (9) (2014) 1794–1805.

[44] M. Haratake, K. Fujimoto, R. Hirakawa, M. Ono, M. Nakayama, Hemoglobin-mediated selenium export from red blood cells, J. Biol. Inorg. Chem. 13 (3) (2008) 471–479.

[45] M. Haratake, K. Fujimoto, M. Ono, M. Nakayama, Selenium binding to human hemoglobin via selenotrisulfide, Biochim. Biophys. Acta 1723 (1–3) (2005) 215–220.

[46] Y. Seko, Y. Saito, J. Kitahara, N. Imura, Active oxygen generation by the reaction of selenite with reduced glutathione in vitro, in: A. Wendel (Ed.), Selenium in Biology and Medicine, Springer Berlin Heidelberg, Berlin, Heidelberg, 1989, pp. 70–73.

[47] A. Ståhl, I. Anundi, J. Høgberg, Selenite biotransformation to volatile metabolites in an isolated hepatocyte model system, Biochem. Pharmacol. 33 (7) (1984) 1111–1117.

[48] A. Demelash, J.O. Karlsson, M. Nilsson, U. Bjorkman, Selenium has a protective role in caspase-3-dependent apoptosis induced by H2O2 in primary cultured pig thyrocytes, Eur. J. Endocrinol. 150 (6) (2004) 841–849.

[49] P. Stiegler, M. Sereinigg, A. Puntschart, A. Bradatsch, T. Seifert-Held, I. Wiederstein-Grasser, B. Leber, E. Stadelmeyer, N. Dandachi, S. Zelzer, F. Iberer, V. Stadlbauer, Oxidative stress and apoptosis in a pig model of brain death (BD) and living donation (LD), J. Transl. Med. (2013) 244.

[50] G. Bermano, F. Nicol, J.A. Dyer, R.A. Sunde, G.J. Beckett, J.R. Arthur, J.E. Hesketh, Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats, Biochem. J. 311 (2) (1995) 425–430.

[51] J.-C. Zhou, H. Zhao, J.-G. Li, X.-J. Xia, K.-N. Wang, Y.-J. Zhang, Y. Liu, Y. Zhao, X.G. Lei, Selenoprotein gene expression in thyroid and pituitary of young pigs is not affected by dietary selenium deficiency or excess, J. Nutr. 139 (6) (2009) 1061–1066.

[52] C.-W. Pyo, S.-H. Lee, S.-Y. Choi, Oxidative stress induces PKR-dependent apoptosis via IFN-γ activation signaling in Jurkat T cells, Biochem. Biophys. Res. Commun. 377 (3) (2008) 1001–1006.

[53] W.T. Wong, X.Y. Tian, Y. Huang, Endothelial dysfunction in diabetes and hypertension: cross talk in RAS BMP4, and ROS-dependent COX-2-derived prostanoids, J. Cardiovasc. Pharmacol. 61 (3) (2013) 204–214.

[54] S.-E. Cheng, I.-T. Lee, C.-C. Lin, W.-L. Wu, L.-D. Hsiao, C.-M. Yang, ATP mediates NADPH oxidase/ROS generation and COX-2/PGE2 expression in A549 cells: role of

P2 receptor-dependent STAT3 activation, PLoS One (2013).
[55] F. Zamamiri-Davis, Y. Lu, J.T. Thompson, K.S. Prabhu, P.V. Reddy, L.M. Sordillo, C.C. Reddy, Nuclear factor-κB mediates over-expression of cyclooxygenase-2 during activation of RAW 264.7 macrophages in selenium deficiency, Free Radic, Biol. Med. 32 (9) (2002) 890–897.
[56] G. Spyrou, M. Björnstedt, S. Skog, A. Holmgren, Selenite and selenate inhibit human lymphocyte growth via different mechanisms, Cancer Res. 56 (19) (1996) 4407–4412.
[57] J. Lu, C. Berndt, A. Holmgren, Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase, Biochim. Biophys. Acta 1790 (11) (2009) 1513–1519.
[58] Y. Ye, Y. Shibata, C. Yun, D. Ron, T.A. Rapoport, A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol, Nature 429 (6994) (2004) 841–847.
[59] M. Marino, T. Stoilova, C. Giorgi, A. Bachi, A. Cattaneo, A. Auricchio, P. Pinton, E. Zito, SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyperoxidation by means of redox-regulating SERCA2 pump activity, Hum. Mol. Gen. 24 (7) (2015) 1843–1855.
[60] Y. Gao, H.C. Feng, K. Walder, K. Bolton, T. Sunderland, N. Bishara, M. Quick, L. Kantham, G.R. Collier, Regulation of the selenoprotein SelS by glucose deprivation and endoplasmic reticulum stress – SelS is a novel glucose-regulated protein, FEBS Lett. 563 (1-3) (2004) 185–190.
[61] I. Cell Biolabs, Product Manual OxiSelect™ Catalase Activity Assay Kit, Colorimetric, Cell Biolabs, Inc., San Diego, CA, USA, 2017.
[62] J. de Groot, G. Kranendonk, M. Fillerup, H. Hopster, W. Boersma, D. Hodgson, K. van Reenen, M. Taverne, Response to LPS in female offspring from sows treated with cortisol during pregnancy, Physiol. Behav. 90 (4) (2007) 612–618.
[63] L.-H. Sun, D.-A. Pi, L. Zhao, X.-Y. Wang, L.-Y. Zhu, D.-S. Qi, Y.-L. Liu, Response of selenium and selenogenome in immune tissues to LPS-induced inflammatory reactions in pigs, Biol. Trace Elem. Res. 177 (1) (2017) 90–96.
[64] W. Manzanares, A. Biestro, F. Galusso, M.H. Torre, N. Manay, G. Pittini, G. Facchin, G. Hardy, Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill? J. Intensive Care Med. 35 (5) (2009) 882–889.
[65] T.K. Vo, P. Godard, M. de Saint-Hubert, G. Morrhaye, C. Swine, V. Geenen, H.J. Martens, F. Debacq-Chainiaux, O. Toussaint, Transcriptomic biomarkers of the response of hospitalized geriatric patients with infectious diseases, Immun. Ageing 7 (2010) 9.
[66] S. Verma, F.W. Hoffmann, M. Kumar, Z. Huang, K. Roe, E. Nguyen-Wu, A.S. Hashimoto, P.R. Hoffmann, Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses, J. Immunol. 186 (4) (2011) 2127–2137.
[67] Z. Huang, F.W. Hoffmann, R.L. Norton, A.C. Hashimoto, P.R. Hoffmann, Selenoprotein K is a novel target of m-calpain, and cleavage is regulated by Toll-like receptor-induced calpastatin in macrophages, J. Biol. Chem. 286 (40) (2011) 34830–34838.
[68] R.L. Norton, G.J. Fredericks, Z. Huang, J.D. Fay, F.W. Hoffmann, P.R. Hoffmann, Selenoprotein K regulation of palmitoylation and calpain cleavage of ASAP2 is required for efficient FcγR-mediated phagocytosis, J. Leukocyte Biol. 101 (2) (2017) 439–448.
[69] N. Petit, A. Lescure, M. Rederstorff, A. Krol, B. Moghadaszadeh, U.M. Wewer, P. Guicheney, Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern, Hum. Mol. Gen. 12 (9) (2003) 1045–1053.
[70] M.L. Tomasi, M. Ryoo, H. Yang, A. Iglesias Ara, K.S. Ko, S.C. Lu, Molecular mechanisms of lipopolysaccharide-mediated inhibition of glutathione synthesis in mice, Free Radic. Biol. Med. 68 (2014) 148–158.
[71] S.W. Kang, S. Lee, E.K. Lee, ROS and energy metabolism in cancer cells: alliance for fast growth, Arch. Pharm. Res. 38 (3) (2015) 338–345.
[72] C.L. Iness, N.B. Metcalfe, The impact of dietary restriction intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish, Proc. Biol. Sci. 275 (1644) (2008) 1703–1708.
[73] K.T. Suzuki, Metabolomics of selenium: se metabolites based on speciation studies, J. Health Sci. 51 (2) (2005) 107–114.
[74] O.M. Radostits, S.H. Done, Diseases associated with nutritional deficiencies – Diseases associated with deficienies of mineral nutrients, in: J. Rodenhuis, R. Demetriou-Swanwick (Eds.), Veterinary Medicine: a Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses, Elsevier Saunders, New York, 2007, pp. 1698–1771.
[75] E. Humann-Ziehank, A. Menzel, P. Roehrig, B. Schwert, M. Ganter, I. Hennig-Pauka, Acute and subacute response of iron zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae, Metallomics 6 (10) (2014) 1869–1879.
[76] M.W. Angstwurm, J. Schottdorf, J. Schopohl, R. Gaertner, Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome, Crit. Care Med. 27 (9) (1999) 1807–1813.
[77] F. Maehira, G.A. Luyo, I. Miyagi, M. Oshiro, N. Yamane, M. Kuba, Y. Nakazato, Alterations of serum selenium concentrations in the acute phase of pathological conditions, Clin. Chim. Acta 316 (1–2) (2002) 137–146.
[78] B. Hollenbach, N.G. Morgenthaler, J. Struck, C. Alonso, A. Bergmann, J. Köhrle, L. Schomburg, New assay for the measurement of selenoprotein P as a sepsis biomarker from serum, J. Trace Elem. Med. Biol. 22 (1) (2008) 24–32.
[79] N.-P. Becker, J. Martitz, K. Renko, M. Stoedter, S. Hybsier, T. Cramer, L. Schomburg, Hypoxia reduces and redirects selenoprotein biosynthesis, Metallomics 6 (5) (2014) 1079–1086.
[80] M. Stoedter, K. Renko, A. Hög, L. Schomburg, Selenium controls the sex-specific immune response and selenoprotein expression during the acute-phase response in mice, Biochem. J. 429 (1) (2010) 43–51.
[81] X. Forceville, V. Mostert, A. Pierantoni, D. Vitoux, P. Le Toumelin, E. Plouvier, M. Dehoux, F. Thuillier, A. Combes, Selenoprotein P rather than glutathione peroxidase, as a potential marker of septic shock and related syndromes, Eur. Surg. Res. 43 (4) (2009) 338–347.
[82] R.F. Burk, K.E. Hill, Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis, Annu. Rev. Nutr. 25 (1) (2005) 215–235.
[83] R.A. Sunde, A.M. Raines, Selenium regulation of the selenoprotein and non-selenoprotein transcriptomes in rodents, Adv. Nutr. 2 (2) (2011) 138–150.
[84] X. Fang, K. Wang, D. Han, X. He, J. Wei, L. Zhao, M.U. Imam, Z. Ping, Y. Li, Y. Xu, J. Min, F. Wang, Dietary magnesium intake and the risk of cardiovascular disease, type 2 diabetes, and all-cause mortality: a dose?response meta-analysis of prospective cohort studies, BMC Med. 14 (1) (2016) 210.
[85] V. Minnich, M.B. Smith, M.J. Brauner, P.W. Majerus, Glutathione biosynthesis in human erythrocytes. I. Identification of the enzymes of glutathione synthesis in hemolysates, Clin. Invest. 50 (3) (1971) 507–513.
[86] L. Yan, L.M. Boylan, J.E. Spallholz, Effect of dietary selenium and magnesium on human mammary tumor growth in athymic nude mice, Nutr. Cancer 16 (3–4) (1991) 239–248.
[87] J.M. Hsu, B. Rubenstein, A.G. Paleker, Role of magnesium in glutathione metabolism of rat erythrocytes, J. Nutr. 112 (3) (1982) 488–496.
[88] R.F. Regan, Y. Guo, Magnesium deprivation decreases cellular reduced glutathione and causes oxidative neuronal death in murine cortical cultures, Brain Res. 890 (1) (2001) 177–183.
[89] J. Sugimoto, A.M. Romani, A.M. Valentin-Torres, A.A. Luciano, C.M. Ramirez Kitchen, N. Funderburg, S. Mesiano, H.B. Bernstein, Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism, J. Immunol. 188 (12) (2012) 6338–6346.
[90] K. Bierla, M. Dernovics, V. Vacchina, J. Szpunar, G. Bertin, R. Lobinski, Determination of selenocysteine and selenomethionine in edible animal tissues by 2D size-exclusion reversed-phase HPLC-ICP MS following carbamidomethylation and proteolytic extraction, Anal. Bioanal. Chem. 390 (7) (2008) 1789–1798.
[91] C. Daun, B. Åkesson, Glutathione peroxidase activity, and content of total and soluble selenium in five bovine and porcine organs used in meat production, Meat Sci. 66 (4) (2004) 801–807.
[92] M.P. Rayman, The use of high-selenium yeast to raise selenium status: how does it measure up? Br. J. Nutr. 92 (4) (2004) 557–573.
[93] E.H. Larsen, J. Sloth, M. Hansen, S. Moesgaard, Selenium speciation and isotope composition in 77Se-enriched yeast using gradient elution HPLC separation and ICP-dynamic reaction cell-MS, J. Anal. At. Spectrom. 18 (4) (2003) 310–316.
[94] P.C. Uden, H. Totoe Boakye, C. Kahakachchi, R. Hafezi, P. Nolibos, E. Block, S. Johnson, J.F. Tyson, Element selective characterization of stability and reactivity of selenium species in selenized yeast, J. Anal. At. Spectrom. 19 (1) (2004) 65–73.
[95] A. Frøslie, J.T. Karlsen, J. Rygge, Selenium in animal nutrition in Norway, Acta Agric. Scand. 30 (1) (1980) 17–25.
[96] D.G. Ellingsen, Y. Thomassen, P. Rustad, P. Molander, J. Aaseth, The time-trend and the relation between smoking and circulating selenium concentrations in Norway, J. Trace Elem. Med. Biol. 23 (2) (2009) 107–115.

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Veterinary medicine

Effects of sodium selenite and L-selenomethionine on feed intake, clinically relevant blood parameters and selenium species in plasma, colostrum and milk from high-yielding sows

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ABSTRACT

A field study in periparturient sows fed different dietary concentrations of either sodium selenite or L-selenomethionine (SeMet) was conducted to evaluate feed intake, haematological and biochemical parameters as well as to describe some key selenium (Se) species, namely selenoprotein P (SelP), selenoalbumin (SeAlb) and selenomethionine (SeMet) as well as total Se in plasma, colostrum and milk.

Thirty-two sows were allotted to four treatments from 30 days (d) prepartum throughout on average a 32 d lactation period. Sodium selenite supplemented diets contained 0.40 and 0.60 mg Se/kg feed, while SeMet supplemented feed contained 0.26 and 0.43 mg Se/kg feed. Concentrations of sodium selenite and SeMet in complete feed exceeded the upper limits for total dietary Se and added organic Se, respectively, according to the European Union legislation. Blood samples were collected at initiation of the study, at farrowing and at weaning. Colostrum samples were collected at farrowing and milk samples at weaning. Se species were subjected to liquid chromatography, and total Se and Se species were determined using inductively coupled plasma mass spectrometry.

The SeMet supplemented diets resulted in higher feed intake and in higher levels of total Se, SelP, SeAlb and SeMet in colostrum compared with sows fed sodium selenite. Similar results were obtained for levels of total Se and SeMet in milk at weaning. The higher dietary sodium selenite concentration in sows' feed did not increase the Se transfer into colostrum or milk when compared with those receiving the lower level of sodium selenite. However, the increase in serum-Zn from initiation until farrowing, observed in sows fed SeMet as well as the higher glutamate dehydrogenase activity in sodium selenite supplemented sows in this period might indicate a higher requirement of antioxidant defence in sodium selenite-supplemented sows.

To our knowledge, the present data on Se species in plasma, colostrum and milk of sows represent the most complete investigation of Se in sows conducted to date. A higher amount of the above-mentioned Se species in the colostrum of sows supplemented with SeMet might strengthen the piglets' antioxidative system and passive immunity as well as improve their average daily weight gain. The higher feed intake in sows fed diets supplemented with SeMet is an interesting finding that warrants further investigation.

1. Background

Sows are exposed to different types of stress as e.g. social stress, heat stress, and oxidative stress during the production cycle [1–4]. Increased systemic oxidative stress in gestation and lactation due to too low levels of compounds with antioxidant function such as selenium (Se) species

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can negatively affect embryonic development, foetal growth and health, the number of stillbirths, litter size as well as postpartum growth of piglets [4–10]. Lower litter size, increased foetal mortality and weak lethargic piglets have been associated with deficiency of Se or vitamin E (vitE) in sows [11]. Supplementation of sows` diets with inorganic or organic Se has been widely discussed [for review see: 12]. According to Finley [13] the physico-chemical forms of Se are generally well transported over the intestinal membrane (70–95 %), but the uptake varies according to the Se source and Se status of the individual. While vitE acts directly, Se exerts its biological functions via selenoproteins that contain selenocysteine (SeCys) in their primary structure, some of which having antioxidant functions [14–18]. Selenoprotein P (SelP) is a selenoprotein, whereas selenoalbumin (SeAlb) contains Se as the selenized amino acid selenomethionine (SeMet), in positions of methionine residues, which is considered to be a “non-specific” form of Se [19]. Adequate feed intake in high yielding sows during lactation can be challenging [20–22]. Optimization of feed composition will improve the sows´ feed intake and thus improve the composition of both colostrum and milk subsequently leading to a better performance of their progeny [23,24]. After birth, the composition of colostrum and milk, also regarding the concentration of Se and selenospecies (Se species) are of remarkable importance to newborns [25–27]. A beneficial antioxidant status in sows could thereby prevent oxidative stress-related effects on the offspring [12,28]. The composition of bioactive molecules in colostrum and milk reflects the nutritional and developmental requirements of mammalian neonates [29].

While there is no limit for vitE, the European Commission (EU) has limited the amount of inorganic Se to a maximum of 0.5 mg total Se/kg feed based on the narrow dose range between Se deficiency and toxicity [30,31]. The addition of organic Se has been confined to 0.2 mg/kg complete feed in the EU to ensure consumer safety [30,32]. Although receiving feed containing Se levels up to 0.5 mg Se/kg feed, nutritional myodegeneration and reproduction related challenges possibly linked to increased oxidative stress due to insufficient Se and/or vitE supply, still occur [33–35]. This raises the question whether actual feeding strategies meet the Se requirements in high-yielding pigs [36,37].

The objectives of the present study were to evaluate effects of different levels of dietary sodium selenite and L-selenomethionine (SeMet) on high-yielding sows´ feed intake and clinically relevant blood parameters as well as on the distribution of selected Se species in plasma, colostrum and milk.

2. Materials and methods

2.1. Study design and animal ethics

This field trial was conducted at a commercial farm and included 32 loose-housed Landrace x Yorkshire sows. The sows were vaccinated against *Erysipelothrix rhusiopathiae*, porcine parvovirus and *Escherichia coli* (Porcilis Ery Parvo vet., MSD Animal health, Netherlands; Neocolipor, Merial, France) according to the manufacturers' recommendations. Until entering the trial, the sows received a diet supplemented with sodium selenite (Retosel®, Se premix 1%, RETORTE Ulrich Scharrer GmbH, Germany) at a level of 0.21 mg Se/kg and selenized yeast (Se yeast; Sel-Plex®, Alltech, USA) at a level of 0.15 mg Se/kg diet. The Se concentration in the unsupplemented baseline diet was 0.04 mg Se/kg feed. The final Se concentration in this diet fed until entering the trial was 0.4 mg Se/kg diet (Format purke soft, Felleskjøpet, Norway).

The duration of the study was two months. About 30 days prior to farrowing, the sows were randomly divided into four groups: NaSe-0.40, NaSe-0.60, SeMet-0.26 and SeMet-0.43 according to the diets described in Table 1. In this article, sodium selenite was abbreviated with NaSe for group names, while SeMet was used for the organic Se source and associated group names as well as for the amino acid selenomethionine itself. Each treatment group comprised two gilts and six sows with two or more parities. Prior to farrowing, the six sows in each group were kept in groups of six in pens of 16.5 m^2 (5.44 m^2 was slatted floor) and the two gilts in pens of 10.3 m^2 (3.36 m^2 was slatted floor). One week *ante partum*, the animals were moved into the farrowing unit and allocated to individual farrowing pens. Within these pens of 7.2 m^2, an area of 1.92 m^2 was slatted floor and the piglet creep area was 1.1 m^2. The sows' diets were supplemented with either sodium selenite (Retosel, Selenium premix 1%, RETORTE Ulrich Scharrer Gmbh, Germany) or SeMet (Excential Selenium 4000, Orffa, Netherlands). The Se concentration in the unsupplemented baseline diet was 0.03 mg Se/kg feed. All diets were added 100 mg vitE/kg feed. The composition of the pelleted feed and its nutritional values are listed in Table 1, and details of feed sampling and determination of total Se content are given in the next sections.

Table 1
Dietary components in and chemical composition of the diets.

Ingredients	% of DM
Barley	30.0
Soy bean	11.3
Wheat	20.0
Wheat bran	7.0
Oats	3.0
Horsebeans	5.0
Pea starch	1.7
Beet pellets	4.0
Animal fat	2.2
Feed lime	1.58
Mono-calcium phosphate	0.56
Feed salt	0.34
Microminerals	0.13
Vitamin A	0.07
Vitamin ADKB	0.07
L-Lysine	0.24
DL-Methionine	0.03
L-Threonine	0.08
L-Tryptophan	0.19
Phyzyme XP 5000 TPT	0.014
Chemical composition (Mean (SD))	
Dry matter (%)	87.12 (0.19)
Water (% of DM)*	12.89 (0.19)
Protein (% of DM)*	15.38 (0.36)
Fat (hydrolysis), (% of DM)*	4.64 (0.30)
Ash (% of DM)*	4.84 (0.11)
Fiber (% of DM)*	4.65 (0.17)
Calcium (% of DM)**	0.94 (0.02)
Phosphor (% of DM)*	0.50 (0.01)
Sodium (% of DM)**	0.24 (0.01)
Trial diet	**Se in mg/kg diet** (Mean (SD))***
NaSe-0.40	0.40 (0.03)
NaSe-0.60	0.60 (0.05)
SeMet-0.26	0.26 (0.04)
SeMet-0.43	0.43 (0.08)

Methods:* Dir. 152/2009/EU; ** ISO 6869; ***ICP-MS from 10 samples per diet.

During the gestation period, the sows were fed twice a day (3.5 kg/day). After farrowing, the sows were fed ad libitum up to four times a day, according to their appetite. The experimental feed was provided manually throughout the trial. Water was provided ad libitum. In the farrowing unit, the room temperature (RT) was set to 20 °C and reduced to 18 °C over a period of one week after farrowing. The trial ended at weaning. Piglets were weaned between day 29 and days 34 of lactation.

The Norwegian Food Safety Authority (application ID 7104) approved the performance of this trial, which complies with the current 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).

2.2. Sampling

Ten feed samples per diet were collected at initiation and end of the trial to determine the total Se content. Blood samples were drawn at initiation of the trial (*Vena jugularis externa*), at farrowing (*Vena subcutanea abdominis*) and at weaning (*V. jug. ext.*). Nine mL Vacuette® Z serum clot activator and six mL Vacuette® Lithium Heparin tubes (Greiner Bio-One, Austria) along with Venoject needles (20 G x 1½"UTW, USA) were used. For analysis, blood samples were centrifuged at 3.500 x *g* for 15 min (Megafuge 1.0 R, Heraeus SEPATECH, USA). Colostrum and milk samples were isolated from three or more teats and pooled. At weaning, an intramuscular injection of 10 IU oxytocin (Vetocin, Bela-Pharm, Germany) was applied to stimulate milk ejection. Feed, serum, plasma, colostrum, and milk samples were stored at -20 °C until analysis.

2.3. Haematology and clinical chemistry

Blood samples were subjected to a complete blood cell count applying a multi-parametric haematological analysis (ADVIA 120 Haematology System, Siemens Healthcare GmbH) with veterinary software. Clinical biochemistry analysis was conducted by applying the ABX Pentra400 analyser (Horiba, France). The analysed variables included glutamate dehydrogenase (GLDH, Roche diagnostics, Norway), as well as ferritin, bilirubin, iron, creatine kinase (CK), aspartate transaminase (AST) and γ-glutamyltransferase (GGT) applying reagents from ABX, Horiba, France. The concentrations of copper (Cu) and zinc (Zn) in plasma were determined using atomic absorption spectroscopy (AA300, Perkin Elmer, USA) at a wavelength of 324.8 and 213.9 nm, respectively. Ceruloplasmin (Cp) was determined by applying the biochemistry analyser Cobas Mira (Roche) using a modification of the method described by Henry et al. [38]. All analyses were conducted at the Norwegian University of Life Sciences (NMBU), Sandnes.

2.4. Selenium in feed, plasma, colostrum and milk

The total Se concentration in feed, plasma, colostrum and milk samples was determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 8800, Japan) after specific sample treatment.

Subsamples, drawn from 10 feed samples per diet, were finely ground and homogenised. An aliquot (~0.25 g) was weighed out directly into Teflon tubes and digested with ultrapure concentrate HNO_3 (5 mL) at 260 °C for 25 min using the UltraClave IV system (Milestone). Digested samples were diluted to 50 mL with ultrapure deionised water and measured directly by ICP-MS. Plasma samples were thawed and treated prior to analysis as described previously by Falk et al. [39], whereas colostrum and milk samples were thawed until reaching room temperature followed by homogenization at 37 °C for 10 min in an ultrasonic bath. Before analysis, subsamples of plasma, colostrum and milk were diluted with a mixture of butanol, EDTA, NH_3 and Triton X-100 (1 + 9 V/V) as described by Liba et al. [40].

Standard solutions were prepared from a Se ICP reference solution (1000 mg L^{-1}, Inorganic Ventures) and ^{74}Se (> 99.9% enriched solution) was added as internal standard to all sample and standard solutions before analysis (except for feed samples where it was added before digestion). ICP-MSMS operating parameters are listed in Table A1 in the supplementary material. Blanks and standard solutions were analysed every 10 samples to monitor signal drift during the run. The carbon effect on the Se signal from the samples was negligible due to the presence of 4% butanol and the use of ^{74}Se as internal standard. Total Se concentrations were calculated from the $^{78}Se/^{74}Se$ signal ratio to account for sample loss during preparation and/or physical interferences. Any contribution from natural ^{74}Se (0.89% isotopic abundance) in the samples and standard solutions to the internal standard was subtracted using a mass bias corrected equation. The quantification limit for Se, calculated as 10 times the standard deviation of the method blanks, was 0.02 µg L^{-1} for plasma, colostrum and milk samples and 0.012 mg kg^{-1} for feed samples.

The accuracy of the methods was controlled by preparing and analysing two NIST standard reference materials (1567a wheat flour and 1570a trace elements in spinach, NIST, USA), two Seronorm™ certified reference materials (Trace elements serum L-1 and L-2, SERO, Norway), and two European reference materials (ERM® BD150 and BD151 skimmed milk powder, JRC, Belgium) in the same manner as feed, plasma and milk samples, respectively. Measured concentrations were within the uncertainties of certified values (Table A2, Supplemental material).

2.5. Selenospecies in plasma, colostrum and milk

The concentration of Se species in plasma, colostrum and milk samples was determined by HPLC–ICP-MSMS. Frozen plasma, colostrum and milk samples were allowed to thaw on ice and homogenized. Plasma (0.5 mL) and colostrum and milk (1 mL) subsamples were transferred into 1.5 mL Eppendorf tubes, and the latter were centrifuged for 60 min at 20,000 *g* and 4 °C. Subsequently, using a fine needle (BD Microlance™ 3, 23 G, 0.6 x 30 mm) and a syringe to minimize contact with the upper fat layer, 0.7 mL of aqueous supernatant was carefully removed and centrifuged at 60,000 *g* for 60 min at 4 °C. Defatted colostrum and milk (supernatant) were collected into new vials. As a final step to all sample types, a 0.5 mL aliquot was centrifuged for 60 min at 14,000 *g* and 4 °C using Amicon 0.5 mL centrifugal filters (3 kDa, Merck, USA). The recovered filtrate and retentate were used for the analysis of low (SeMet) and high (SelP, SeAlb) molecular weight Se species, respectively.

All species were separated and quantified using 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 (TRA). Three different column set-ups were used under isocratic elution conditions (1-1.5 mL min^{-1}). The free amino acid SeMet in the < 3 kDa filtrate fractions was determined (10 µL injection volume) using an Agilent Poroshell 120 column (SB-C18, 3 x 75 mm, 2.7 µm) with a mobile phase of 5:95 methanol:water with 0.1% (V/V) of heptafluorobutyric acid (HFBA). Both SelP and SeAlb were determined from retentate fractions (50 µL injection volume) using, respectively, 1 mL HiTrap Heparin and HiTrap Blue HP columns (GE Healthcare, Uppsala, Sweden) with 0.05 M and 1.5 M ammonium acetate solutions as mobile phases (named "A" and "B", respectively). The retention-elution strategies were as follows: 100% A from 0 to 2.50 min (HiTrap Heparin) and 0.20 min (HiTrap Blue), and 100% B from 2.51 to 7 min (HiTrap Heparin) and from 0.21 to 10 min (HiTrap Blue). The ICP-MSMS operating parameters to measure ^{78}Se (Table A1) and examples of chromatograms for representative plasma, colostrum and milk samples from pigs of two different diets (Figures A1 and A2) are given in the supplementary material. Although all peaks in the chromatograms were integrated during data treatment, only areas of peaks associated to known Se species, eluting at the correct retention time (t_R; i.e., t_R ~ 6.5 min for SeMet and t_R ~ 4-4.5 min for SelP and SeAlb) were considered for quantification. Since no SelP or SeAlb commercial compounds were available at the time of analysis, seleno-L-methionine (≥98% (TLC), Sigma Aldrich) was used to prepare SeMet standard solutions (1–10 and 50–500 µg Se L^{-1} for Agilent and HiTrap column methods, respectively) and to obtain a calibration curve (i.e., peak area vs. Se concentration) under the specific analysis conditions of each method. Even if SeMet is not retained in the HiTrap columns (t_R ~ 1 min) and hence differs in elution time from SelP and SeAlb, isocratic elution conditions (i.e., no changes in mobile phase composition throughout the elution of Se species) enabled the use of SeMet calibration curves for the quantification of the three Se species, i.e., SelP, SeAlb and SeMet. Cross contamination and potential carryover were assessed by running blank samples (vials with mobile phase and washing solutions) every 10 samples, and no ghost or carryover peaks

were detected.

2.6. Statistical analysis

Initially, data from 32 sows were obtained. Two sows were excluded from the trial because of disease (see *Results*). Data from these two sows were removed from the data set. Statistical analyses were performed in RStudio, Version 1.1.383 - © 2009–2017 RStudio, Inc. [42].

To compare the relations between Se and some Se species in colostrum and milk versus that in plasma of all four groups of sows, colostrum-Se/plasma-Se and milk-Se/plasma-Se ratios were calculated. In addition to measurements of various parameters throughout the trial, a Cp/Cu-ratio was calculated. Values obtained by haematology and clinical chemistry, absolute amounts of Se and Se species in plasma, colostrum (farrowing) and milk (weaning) as well as the calculated ratios were modelled using a linear model (R *lm*). The explanatory variable *Diet* was modelled as four different diets.

Feed intake was modelled using a mixed effect model where the explanatory variables *Diet* and *Time after farrowing* were included as fixed effects and *Sow* as random effect, allowing each sow to influence the model at an individual level. The continuous variable *Time after farrowing* was modelled as a nonlinear function using spline in a gam model (R library mgcv). Prediction of mean feed intake for each diet with confidence interval (95% CI) was calculated from the final model for each day after farrowing and plotted to visualize differences between diets. The proportion of the variance accounted for by the random effect (sow specific effect) was calculated as the intraclass correlation coefficient (ICC), which is equal to the variance of the random effect divided by variance of the random effect plus variance of the residuals [43]. Based on the average daily feed intake (ADFI), the Se intake/day and sow was calculated. The explanatory variables regarding genetics (sows' genetic/ genetic of father/ genetic of mother) were included.

Plasma-Se concentrations were correlated with colostrum- and milk-Se concentration.

The significance level was set to $P < 0.05$.

3. Results

One week ante partum, one of the sows in group SeMet-0.26 was euthanized due to septic arthritis causing substantial pain and severe lameness. One sow fed NaSe-0.40 was excluded from the experiment the first day after farrowing due to peracute mastitis-metritis-agalactia causing pyrexia, agalactia and anorexia. Piglets originating from this sow were allocated to other sows within the same treatment group. There was no difference in litter size between groups.

3.1. Dietary selenium content and feed intake

Dietary Se levels were determined to be as followed: 0.40 (0.03) mg/kg feed [NaSe-0.40], 0.60 (0.05) mg/kg feed [NaSe-0.60], 0.26 (0.04) mg/kg feed [SeMet-0.26] and 0.43 (0.08) mg/kg feed [SeMet-0.43], respectively.

The mean lactation period lasted 31.5 ± 0.2 days. Over this period, an effect on ADFI was observed (Fig. 1). From day 13 *post partum*, the ADFI became higher in the sows fed SeMet supplemented diets ($P < 0.001$) compared with those supplemented with sodium selenite. Sows fed NaSe-0.40 and NaSe-0.60 reached an ADFI peak at 6.47–6.69 kg feed/day on day 21 and showed subsequent ADFI decrease. However, the ADFI of sows supplemented with SeMet increased almost steadily from day 13 (6.40–6.62 kg feed/day) until the end of lactation (7.47–7.86 kg feed/day).

The mean total amounts of feed consumed in the lactation period (95% CI) in groups fed NaSe-0.40 and NaSe-0.60, and SeMet-0.26 and SeMet-0.43 were 174.1 kg (169.4–178.7 kg), 176.8 kg (172.3–181.3 kg), 195.5 kg (190.8–200.1 kg) and 191.6 kg (187.2–196.1 kg), respectively. On average, ADFI was 0.88 (0.32) kg/day higher in sows given SeMet-0.26 and SeMet-0.43 compared with those receiving NaSe-0.40 and NaSe-0.60. During the whole lactation period, sows fed SeMet-0.26 received significantly less Se than the other groups, whereas sows given NaSe-0.60 were fed the highest amounts of Se ($P < 0.001$, data not shown). Different Se-levels of the same Se source did not influence the ADFI.

3.2. Haematology, clinical biochemistry and microminerals

The haematological and biochemical parameters showing significant differences between groups are summarized in Table 2. Non-significant differences were observed for most studied variables (Tables A3-A6, Supplementary material).

In the period from initiation to farrowing, the GLDH activity increased more in sows provided with NaSe compared with sows fed SeMet-supplemented diets. From farrowing to weaning, the GLDH activity of sows fed SeMet-0.43 increased significantly compared with sows in the other groups. From initiation to farrowing, the enzyme-activity of GGT increased more in sows fed NaSe-0.40 (trend vs. SeMet-0.4: $P = 0.06$) and SeMet-0.26 than in those given NaSe-0.60 and SeMet-0.43. In the same period, the serum Zn-levels tended to increase more in sows fed SeMet-0.43 compared with sows given NaSe-0.40 ($P = 0.05$). Furthermore, the Cp/Cu- ratio decreased more from farrowing to weaning in groups fed sodium selenite versus those fed SeMet.

3.3. Total selenium in plasma, colostrum and milk

Total Se concentrations in plasma, colostrum and milk are shown in Fig. 2. The initial plasma Se concentration was 182 ± 7 μg L^{-1} (mean ± SEM). Total plasma Se increased from farrowing to weaning but there was no significant difference for this parameter between groups during the trial. The total Se concentrations in colostrum and milk tended to increase with the feed concentration of SeMet. The Se concentrations in colostrum and milk from sows fed SeMet were significantly higher than from sows fed NaSe-0.40, and no significant difference was found between the groups fed NaSe-0.40 and NaSe-0.60. This is also shown by the standardised Se concentrations in colostrum and milk towards Se concentrations in plasma (ratio colostrum Se/plasma Se and milk Se/plasma Se; Table 3).

3.4. Selenospecies in plasma, colostrum and milk

Similarly as for total Se, the SelP concentration in plasma showed no dietary effect, but increased in colostrum with SeMet in feed (Fig. 2). However, SelP levels were low in milk without group differences. SeAlb in plasma was found to be influenced by Se type and concentrations in feed, showing low concentrations in sows fed SeMet-0.26 at farrowing and weaning, and in sows given NaSe-0.40 at weaning. In colostrum, SeAlb increased clearly with feed concentration of SeMet, but low concentrations without group differences were found in milk. SeMet was not detectable in plasma but increased with increasing feed SeMet concentration in colostrum and milk. Higher levels of SeMet were observed in milk than in colostrum.

Evidently as demonstrated by the calculated ratios, transfer of SelP and SeAlb, and thus total Se, to colostrum (farrowing) was much higher than to milk at weaning (Table 3, *P < 0.001*). Feeding of diets added SeMet led to proportionally higher ratios of SelP and SeAlb at farrowing. Interestingly, despite an even greater difference in the dietary Se dose, the ratios for SelP and SeAlb at farrowing were similar for the two inorganic Se diets. In the *Discussion* section, detected Se species were divided in low (< 3 kDa, free amino acid SeMet) and high (> 3 kDa, SelP and SeAlb proteins) molecular mass species, with the latter containing Se bound in their amino acid chain.

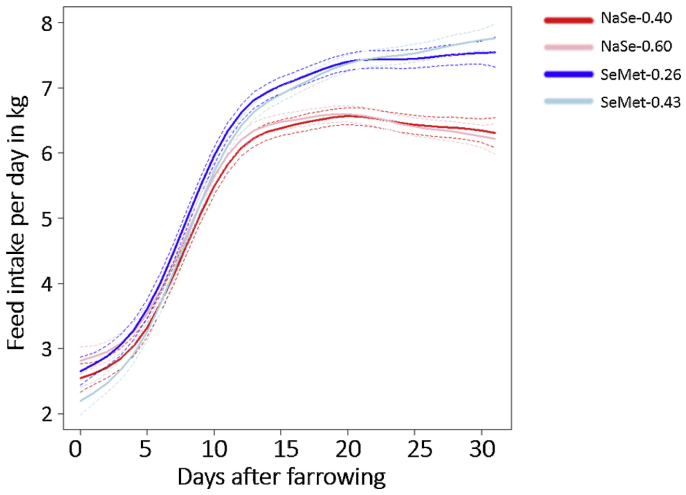


Fig. 1. Effect of time and diet on feed intake (kg/day ± 95% confidence interval).
Feed intake of sows throughout the lactation period. The variance of the residuals in this gamm model was 0.93. ICC = 0.346. During the lactation period, a dietary effect on average daily feed intake (ADFI) was observed. From day 13 post partum until the end of the study, the ADFI was significantly higher in the SeMet supplemented SeMet-0.26 and SeMet-0.43 compared with the sodium selenite supplemented NaSe-0.40 and NaSe-0.60 ($P < 0.001$). Both SeMet-0.26 and SeMet-0.43 showed a continuing increase in ADFI whereas NaSe-0.40 and NaSe-0.60 reached an ADFI peak with subsequent decrease.
NaSe-0.40: sodium selenite 0.40 mg Se/kg diet; NaSe-0.60: sodium selenite 0.60 mg Se/kg diet; SeMet-0.26: L-selenomethionine 0.26 mg Se/kg diet; SeMet-0.43: L-selenomethionine 0.43 mg Se/kg diet.

Table 2
Blood parameters in sows (Mean (SD)).

Parameter	Initiation[1]	Change over time (%)		
		I-F	F-W	I-W
Enzyme activities				
GGT (U/L)				
NaSe-0.40	52.1 (15.0)	−30.5 (10.1)	64.9 (26.9)^{ab}	13.7 (19.1)$^{ab\hat{}\hat{}}$
NaSe-0.60	67.0 (18.7)	−24.4 (9.7)	29.8 (25.3)^{a}	−7.4 (17.7)^{a}
SeMet-0.26	62.4 (18.3)	−28.4 (16.2)	81.8 (66.5)^{b}	23.2 (30.3)^{b}
SeMet-0.43	63.5 (16.7)	−30.9 (7.7)	34.4 (15.3)^{a}	−7.6 (10.8)$^{a\hat{}\hat{}}$
GLDH (U/L)				
NaSe-0.40	1.9 (0.4)	50.0 (76.4)^{bc}	14.3 (24.4)^{a}	64.3 (69.0)
NaSe-0.60	1.0 (0.6)	90.0 (74.2)^{b}	6.3 (80.6)^{a}	80.0 (83.7)
SeMet-0.26	2.1 (0.7)	21.4 (39.3)^{ac}	11.9 (38.1)^{a}	38.1 (77.4)
SeMet-0.43	2.3 (0.5)	−20.8 (23.1)^{a}	93.8 (72.9)^{b}	39.6 (17.7)
Microminerals related results				
Cp/Cu				
NaSe-0.40	3.5 (0.6)	−23.7 (11.8)	−15.0 (19.9)^{ab}	13.0 (26.3)
NaSe-0.60	3.6 (0.5)	−20.4 (4.7)	−21.6 (16.2)^{a}	−1.7 (15.9)
SeMet-0.26	3.4 (0.6)	−30.3 (13.2)	−8.5 (16.8)^{b}	35.1 (33.0)
SeMet-0.43	3.5 (0.5)	−26.8 (5.7)	−4.2 (19.3)^{b}	31.5 (28.1)
Zn (μmol/L)				
NaSe-0.40	10.0 (1.0)	−9.0 (15.5)$^{a\hat{}}$	17.4 (15.1)	17.4 (15.1)
NaSe-0.60	10.3 (1.2)	−1.3 (16.6)^{ab}	18.3 (10.3)	18.3 (10.3)
SeMet-0.26	10.4 (1.5)	4.3 (19.3)^{ab}	33.5 (18.5)	33.5 (18.5)
SeMet-0.43	9.7 (1.5)	15.8 (33.6)$^{b\hat{}}$	31.3 (28.7)	31.3 (28.7)

Only parameters showing significant inter-group differences are presented. [1]Initiation: Measured values. I–F: Change from initiation to farrowing, F−W: Change from farrowing to weaning; I−W: Change from initiation to weaning; GGT - γ-glutamyl transferase, GLDH – glutamate dehydrogenase, Cp/Cu – ceruloplasmin/copper ratio, Zn – zinc; Means within a column without a common superscript differ significantly ($P < 0.05$). ^P = 0.05, ^^P = 0.06.
NaSe-0.40: sodium selenite 0.40 mg Se/kg diet; NaSe-0.60: sodium selenite 0.60 mg Se/kg diet; SeMet-0.26: L-selenomethionine 0.26 mg Se/kg diet; SeMet-0.43: L-selenomethionine 0.43 mg Se/kg diet.

4. Discussion

The difference in dietary Se levels between 0.43 (group SeMet-0.43) and 0.40 (group NaSe-0.40) is within the uncertainty of the analytical method and considered numerically comparable.

4.1. Feed intake

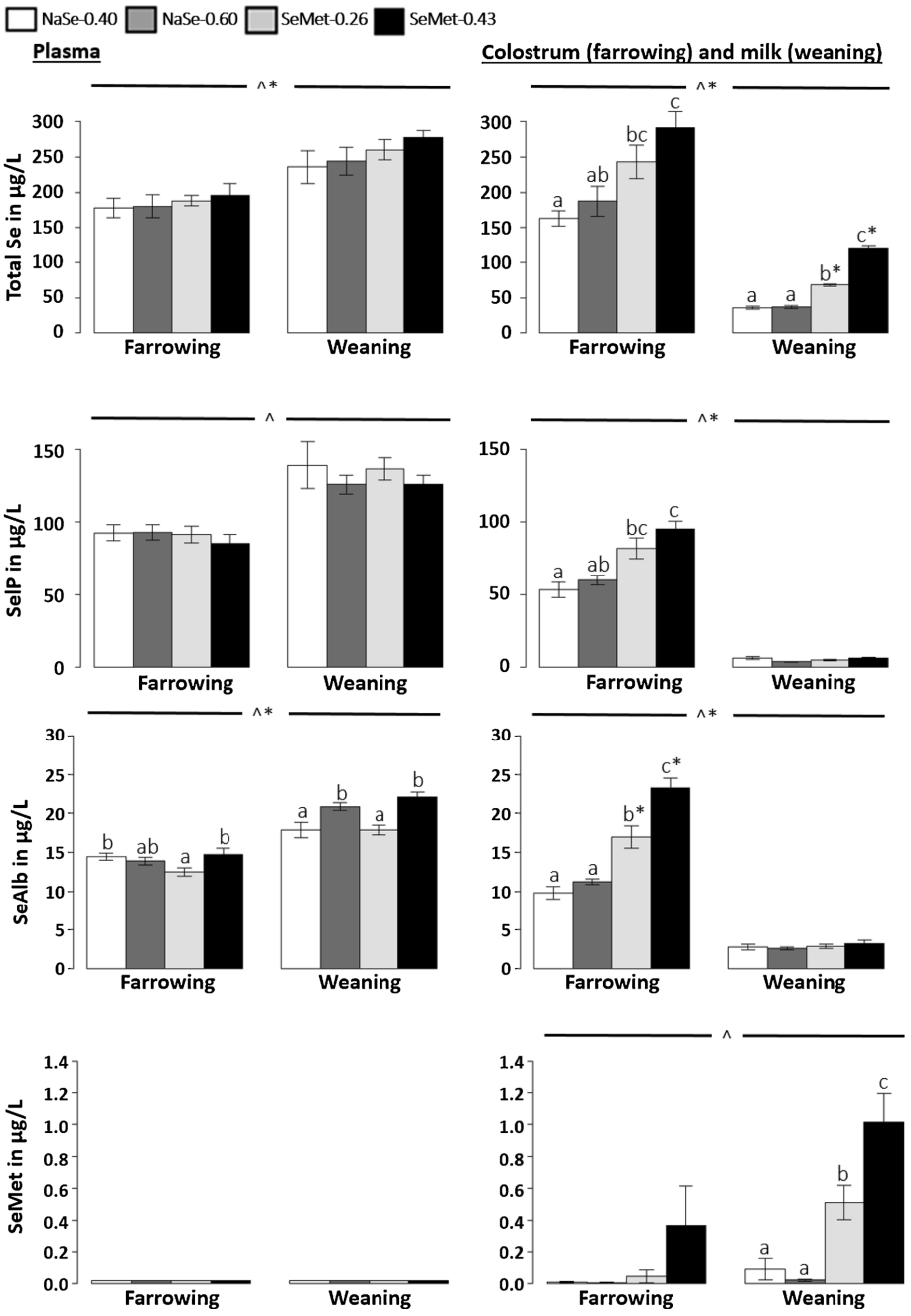
The reason for the higher ADFI observed in SeMet supplemented sows compared with those fed NaSe-0.40 and NaSe0.60 remained uncertain. It could be related to the smell of the feed. The farmer and researchers had a clear perception of different odours of the sodium selenite supplemented diets versus those supplemented with SeMet. The olfactory system of pigs is highly developed with quite large and highly organized structures [44,45]. As smell is an initial attractant to feed, the sows fed SeMet may have consumed more feed due to an appealing smell perception. Jankevicius and Widowski [46] suggested that pigs decide whether to consume offered feed or not according to their olfactory or taste perception. Taheri et al. [47] observed a higher feed intake in goats fed organic Se versus those fed sodium selenite in a 45 days feeding trial conducted after parturition, which is in line with our findings.

The Norwegian Landrace x Yorkshire hybrid used in this study is a very lean and feed efficient sow [48] being more prone to nutritional stress, especially during lactation, compared with sows that have higher levels of body reserves [49]. Maintenance requirements of contemporary modern lean genotypes are higher due to the increased needs for energy to maintain lean muscle tissue compared with adipose tissue [50,51]. Sows fed SeMet-0.26 and SeMet-0.43 consumed more feed over a period of more than 2 weeks, in theory enabling them to maintain or re-establish their body condition for the subsequent reproductive cycle

4.2. Clinical biochemistry

From initiation until farrowing, the increase in serum-Zn observed in sows fed SeMet than in those receiving NaSe-0.40 may relate to its antioxidant function. Zn decreases when oxidative stress levels increase [52]. In the same period, the enzymatic activity of GLDH increased stronger in sodium selenite supplemented sows compared with those fed SeMet. GLDH controls the intracellular levels of fumarate, which binds to and activate glutathione peroxidase 1, thus regulating redox homeostasis [53]. This might indicate a higher requirement of antioxidant defence in sodium selenite-supplemented sows.

During lactation, a stronger increase of GGT activity was observed in sows fed NaSe-0.40 and SeMet-0.26 compared with those fed higher levels of sodium selenite and SeMet, respectively. This could be related to the metabolism of glutathione, where GGT catalyses the first step of



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the recycling process. Serum and dietary antioxidant vitamins have been shown by others to have inverse dose response relations to serum GGT level within its normal range [54].

SeMet-supplementation may have allowed maintenance of a stable antioxidative status in sows without the need to compensate by applying alternative antioxidative molecules like Zn-containing enzymes when experiencing elevated systemic oxidative stress levels, e.g. during late gestation and lactation [4]. The stronger increasing Cp/Cu-ratio from farrowing to weaning in groups fed SeMet versus those fed sodium selenite might indicate a smaller pool of free copper ions in the

Fig. 2. Levels of total selenium in plasma, colostrum and milk compared with selenospecies (µg/L ± 95% confidence interval).
Colostrum and milk samples isolated from SeMet-0.26 and SeMet-0.43 showed higher selenium (Se) than NaSe-0.40 and/or NaSe-0.60. Colostrum contained much higher amounts of Se compared with milk. Selenoprotein P- (SelP)- and selenoalbumin- (SeAlb)-levels in plasma were highest in samples isolated at weaning. Milk-levels of SelP and SeAlb, where lower compared with those in colostrum. The concentration of SeAlb in milk seemed to correlate with the dietary Se source.
Selenomethionine (SeMet) was not detected in plasma (graph shows LOD = 0.02 µg/L). In colostrum samples, SeMet was only detected with organic Se diets. In milk samples, the concentration of SeMet was higher in SeMet-0.43 sows than with SeMet-0.26. Milk-SeMet levels appear to correlate with the level of SeMet supplementation.
Bars at one time point without a common superscript differ significantly ($P < 0.05$). The symbol ˆ between time points notifies significant difference ($P < 0.05$); *$P < 0.001$
NaSe-0.40: sodium selenite 0.40 mg Se/kg diet; NaSe-0.60: sodium selenite 0.60 mg Se/kg diet; SeMet-0.26: L-selenomethionine 0.26 mg Se/kg diet; SeMet-0.43: L-selenomethionine 0.43 mg Se/kg diet.

Table 3
Calculated ratios (colostrum/plasma [farrowing] and milk/plasma [weaning]) for total selenium and related species.

Group	Time point	Ratio Total Se	Ratio SelP	Ratio SeAlb
NaSe-0.40	Farrowing	0.93 (0.20)^{a}	0.59 (0.15)^{a}	0.69 (0.17)^{a}
NaSe-0.60	Farrowing	1.26 (0.30)^{ab}	0.66 (0.17)^{a}	0.81 (0.10)^{a}
SeMet-0.26	Farrowing	1.40 (0.27)^{ab}	0.92 (0.28)^{b}	1.36 (0.26)^{bc}
SeMet-0.43	Farrowing	1.59 (0.52)^{b}	1.15 (0.27)^{b}	1.57 (0.32)^{c}
NaSe-0.40	Weaning	0.16 (0.04)^{a}	0.05 (0.02)^{ab}	0.16 (0.06)^{a}
NaSe-0.60	Weaning	0.15 (0.05)^{a}	0.03 (0.00)^{a}	0.13 (0.02)^{a}
SeMet-0.26	Weaning	0.28 (0.04)^{b}	0.03 (0.01)^{ab}	0.14 (0.02)^{a}
SeMet-0.43	Weaning	0.43 (0.02)^{c}	0.05 (0.01)^{b}	0.15 (0.03)^{a}

The table shows the above−mentioned ratios for the concentrations of total selenium (Total Se), selenoprotein P (SelP) and selenoalbumin (SeAlb) as mean (SD). Means within a column and at the same time point denoted with different superscripts differ significantly ($P < 0.05$). ˆ$P = 0.05$.
NaSe-0.40: sodium selenite 0.40 mg Se/kg diet; NaSe-0.60: sodium selenite 0.60 mg Se/kg diet; SeMet-0.26: L-selenomethionine 0.26 mg Se/kg diet; SeMet-0.43: L-selenomethionine 0.43 mg Se/kg diet.

organic Se supplemented sows. In humans, non-Cp copper levels correlate negatively with antioxidants and positively with free radical products, conforming the pro-oxidant role of intracellular free copper [55].

Our results on GGT, GLDH and on the Cp/Cu-ratio could indicate a reaction of the body on the increased level of oxidative stress caused by dietary sodium selenite in addition to the reproduction-induced stress. A study in grower-finisher pigs indicated that supplementing with sodium selenite increases the levels of stress after exposure to LPS compared with pigs supplemented with organic Se sources [39].

4.3. Total Se in plasma, colostrum and milk

The observation of increasing Se levels in sows' plasma from farrowing to weaning is in line with a study by Yoon and McMillan [56], who observed a gradual increase during lactation. During late gestation, the blood volume increases leading to hemodilution [57], possibly improving placental microcirculation to accelerate foetal development [58]. Blood and serum volumes normalize again after farrowing [57], which might explain the observed lower plasma Se levels at farrowing.

Higher protein levels in colostrum versus milk [59–61] explain the higher Se concentrations in colostrum compared with milk obtained at weaning. In line with previous studies [62–66] we observed higher total Se levels in colostrum and milk from sows fed SeMet enriched diets compared with sows fed sodium selenite. Consistent with results from previous studies in sows [62,63] and cows [65], diet showed a dose-response effect on milk Se in sows given SeMet. Some of this increase in milk-Se in SeMet-fed sows versus sodium selenite-fed sows could have been associated with the higher ADFI, but colostrum- and milk- Se levels did not reflect the total Se-intake per day and sow during the lactation period. The daily total Se-intake was lowest in sows fed SeMet-0.26, and highest in those provided with NaSe0.60 of all groups (data not shown). Milk from sows in the groups fed NaSe-0.40 and NaSe-0.60 showed similar Se levels, despite different dietary sodium selenite-levels. Our results correlate well with previous studies indicating higher bioavailability and higher ability of organic Se versus inorganic Se to increase Se in colostrum and milk [62–68]. Mahan [62] calculated that, with an assumed daily milk production in sows of 10 kg/d, approximately 30% of the inorganic and 80% of the organic dietary Se would be excreted with the milk. However, Mahan and Kim [63] could not find any influence of neither Se-source nor dietary Se levels on Se in colostrum. In that study, however, Se yeast was used as organic Se-source, and recent literature has shown that there may be a large batch-to-batch and product-to-product variability of the SeMet content in Se yeast [69].

Higher Se concentrations in colostrum have shown to increase the passive absorption of immunoglobulin G in calves [70]. Kielland et al. [71] suggested that improved levels of IgG in piglets potentially increase the survival of piglets. Based on the significant higher milk/plasma-ratio for Se in sows fed SeMet one could suggest that they received a Se-source and dietary level covering their demands, and simultaneously allowing a higher transfer of Se to the mammary gland. In contrast to sodium selenite, SeMet can be stored in the body´s protein pool and is continuously available by protein turnover enabling a Se homeostasis.

4.4. High molecular mass Se species in plasma, colostrum and milk

Overall, the concentrations of SelP, the major Se transport protein to the mammary gland secretions [72], and SeAlb showed a similar trend compared with the total Se levels in both plasma and colostrum. SelP [72] and SeAlb [73,74] are transferred from extramammary tissue to colostrum and milk. Olson et al. [75] showed a receptor –mediated uptake for SelP via the megalin receptor in kidney cells. Rowling et al. [76] showed that megalin was expressed by human mammary derived cells. An active, receptor-mediated transport mechanism could explain our results on SelP-levels in colostrum and milk versus plasma. Colostrum- /plasma- and a milk- /plasma- ratios were calculated for SelP and SeAlb, implying that the transfer of SelP and SeAlb, and thus that of Se in total, is higher to colostrum than to milk. Thus, in opposite to de la Flor et al. [77], who examined human colostrum and milk during the first month after delivery, the distribution patterns in the sows´ mammary gland secretions changed throughout lactation.

In line with observations made by Fantuz et al. [78] in donkeys, our results indicate that the mammary gland actively regulates the transport of Se. Milk levels of SelP and SeAlb and thus the total Se concentration in milk decreased as lactation progressed possibly by active reduction of their transport. However, Lönnerdal [79] suggested that human milk Se levels are closely correlated with circulating Se levels, making a regulating mechanism unlikely. Our results cannot support the statement made by Lönnerdal [79], which might reflect the different nutritional and developmental requirements of mammalian neonates [29].

4.5. Low molecular mass (< 3 kDa) Se species in plasma, colostrum and milk

Muñiz-Naveiro et al. [80] detected three Se species in milk from

cows supplemented with Se yeast (SeCys, selenite and SeMet), whereas only SeCys and selenite in milk from cows supplemented with sodium selenite. Milk levels of SeMet detected in our trial are comparable to those found in cow milk [81]. Free amino acids in milk have been shown by others to increase throughout lactation in sows [82], which is in line with our observations.

A lack of Se or individual selenoproteins has been shown to result in growth retardation in young mice and human [25]. Thus, the higher level of the selenized amino acid SeMet, in milk compared with colostrum may relate to the high growth rate and the resulting high metabolism of the newborns.

Our calculations showed that SelP, SeAlb and SeMet comprised about 57% (at farrowing) and 62% (at weaning) of total Se in the sows' plasma. As shown by others, Se in plasma is mainly bound to SelP (~50%, [83,84]) and glutathione peroxidase 3 (Gpx3; 4 30%; [84–86]). However, our results on Se speciation comprise only about 34% of total Se in colostrum and 13% in milk at weaning. In the present study, the levels of GPx3 were unfortunately not determined in the sows' plasma, colostrum nor milk due to the lack of methodological requirements.

Fat has been removed from colostrum and milk prior to Se speciation, but was shown to be quite low in Se. Regardless of species (cow, sheep, goat, human) < 3-5% of total Se in milk were associated with the lipid fraction [85,87]. In contrast to plasma Se (< 3%), a large fraction of milk Se, approximately 30%, is present in small-molecule forms of Se not yet characterized [85,88]. In addition, SeMet enters proteins non-specifically and sodium selenite provides Se only for selenoproteins [72], partly explaining the differing fractions of Se from colostrum and milk in our study. Bierla et al. [89] showed that most of the total Se, over 70%, was present in the casein fraction.

Occurrence and concentrations of SelP, SeAlb and SeMet have not been described earlier in plasma, colostrum and milk from sows. Knowledge of the fate of these molecules during gestation and lactation is important in order to understand their significance and contribution to animal health and to provide a basis for further research.

4.6. Conclusion

Organic Se was superior to inorganic Se when comparing sows´ feed intake as well as transfer of total Se and the main Se transport protein SelP from plasma to colostrum and milk. In addition, we suggest that substitution of dietary sodium selenite with SeMet might decrease oxidative stress in highly prolific sows as indicated by serum-Zn, GLDH and GGT. Elevated concentrations of selected Se species as detected in colostrum from sows supplemented with SeMet compared with those fed sodium selenite potentially improve the piglets´ survival and growth due to increased uptake of IgG and probably better antioxidative status. The significant lower levels of total Se, SelP and SeAlb found in milk at weaning versus colostrum may be consistent with an active regulation of the Se transport from extramammary tissue to colostrum and milk. Future research should focus on the influence of different Se sources on blood parameters as well as on the Se transfer in the mammary gland throughout lactation and on subsequent effects on production parameters of the progeny.

Conflict of interest

None.

Acknowledgements

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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.2018.12.009.

References

[1] C. Munsterhjelm, A. Valros, M. Heinonen, O. Hälli, O.A.T. Peltoniemi, Housing during early pregnancy affects fertility and behaviour sows, Reprod. Domest. Anim. 43 (5) (2008) 584–591.
[2] M. Mendl, A.J. Zanella, D.M. Broom, Physiological and reproductive correlates of behavioural strategies in female domestic pigs, Anim. Behav. 44 (6) (1992) 1107–1121.
[3] V.T. Tsuma, S. Einarsson, A. Madej, N. Lundeheim, Cortisol and β-endorphin levels in peripheral circulation around weaning in primiparous sows, Anim. Reprod. Sci. 37 (2) (1995) 175–182.
[4] C.B. Berchieri-Ronchi, S.W. Kim, Y. Zhao, C.R. Correa, K.J. Yeum, A.L.A. Ferreira, Oxidative stress status of highly prolific sows during gestation and lactation, Animal 5 (11) (2011) 1774–1779.
[5] I.T. Omtvedt, R.E. Nelson, R.L. Edwards, D.F. Stephens, E.J. Turman, Influence of heat stress during early, mid and late pregnancy of gilts, J. Anim. Sci. 32 (2) (1971) 312–317.
[6] D. Renaudeau, J. Noblet, Effects of exposure to high ambient temperature and dietary protein level on sow milk production and performance of piglets, J. Anim. Sci. 79 (6) (2001) 1540–1548.
[7] L.J. Johnston, M. Ellis, G.W. Libal, V.B. Mayrose, W.C. Weldon, Effect of room temperature and dietary amino acid concentration on performance of lactating sows. NCR-89 Committee on Swine Management, J. Anim. Sci. 77 (7) (1999) 1638–1644.
[8] A.M. Williams, T.J. Safranski, D.E. Spiers, P.A. Eichen, E.A. Coate, M.C. Lucy, Effects of a controlled heat stress during late gestation, lactation, and after weaning on thermoregulation, metabolism, and reproduction of primiparous sows, J. Anim. Sci. 91 (6) (2013) 2700–2714.
[9] E.C. Cottrell, J.R. Seckl, Prenatal stress, glucocorticoids and the programming of adult disease, Front. Behav. Neurosci. 3 (2009) 1–9.
[10] Y. Zhao, W.L. Flowers, A. Saraiva, K.-J. Yeum, S.W. Kim, Effect of social ranks and gestation housing systems on oxidative stress status, reproductive performance, and immune status of sows, J. Anim. Sci. 91 (12) (2013) 5848–5858.
[11] H. Wuryastuti, H.D. Stowe, R.W. Bull, E.R. Miller, Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum, and milk leukocytes of sows, J. Anim. Sci. 71 (9) (1993) 2464–2472.
[12] P.F. Surai, V.I. Fisinin, Selenium in sow nutrition, Anim. Feed Sci. Technol. 211 (2016) 18–30.
[13] J. Finley, Bioavailability of selenium from foods, Nutr. Rev. 64 (3) (2006) 146–151.
[14] J.M. Finch, R.J. Turner, Effects of selenium and vitamin E on the immune responses of domestic animals, Res. Vet. Sci. 60 (2) (1996) 97–106.
[15] M. Birringer, S. Pilawa, L. Flohe, Trends in selenium biochemistry, Nat. Prod. Rep. 19 (6) (2002) 693–718.
[16] L.V. Papp, J. Lu, A. Holmgren, K.K. Khanna, From selenium to selenoproteins: synthesis, identity, and their role in human health, Antioxid. Redox Signal. 9 (7) (2007) 775–806.
[17] V. Pagmantidis, C. Meplan, E.M. van Schothorst, J. Keijer, J.E. Hesketh, Supplementation of healthy volunteers with nutritionally relevant amounts of selenium increases the expression of lymphocyte protein biosynthesis genes, Am. J. Clin. Nutr. 87 (1) (2008) 181–189.
[18] H. Steinbrenner, H. Sies, Protection against reactive oxygen species by selenoproteins, Biochim. Biophys. Acta 1790 (11) (2009) 1478–1485.
[19] R.F. Burk, K.E. Hill, A.K. Motley, Plasma selenium in specific and non-specific forms, Biofactors 14 (1-4) (2001) 107–114.
[20] D.K. Revell, I.H. Williams, Physiological control and manipulation of voluntary feed intake, in: E.S. Batterham (Ed.), Manipulating Pig Production (Australasian Pig Science Association), 1993, pp. 55–80 Werribee.
[21] J.J. Eissen, E. Kanis, B. Kemp, Sow factors affecting voluntary feed intake during

lactation, Livest. Prod. Sci. 64 (2–3) (2000) 147–165.
[22] S.L. Thingnes, A.H. Gaustad, N.P. Kjos, E. Sandberg, T. Framstad, The effect of different dietary energy levels during rearing and mid-gestation on sow lifetime performance and longevity, Acta Agric. Scand. Sect. A — Anim. Sci. 65 (3-4) (2015) 148–157.
[23] C. Lauridsen, V. Danielsen, Lactational dietary fat levels and sources influence milk composition and performance of sows and their progeny, Livest. Prod. Sci. 91 (1) (2004) 95–105.
[24] U. Krogh, T.S. Bruun, C. Amdi, C. Flummer, J. Poulsen, P.K. Theil, Colostrum production in sows fed different sources of fiber and fat during late gestation, Can. J. Anim. Sci. 95 (2) (2015) 211–223.
[25] A.K. Rundlöf, E.S. Arnér, Regulation of the mammalian selenoprotein thioredoxin reductase 1 in relation to cellular phenotype, growth, and signaling events, Antioxid. Redox Signal. 6 (1) (2004) 41–52.
[26] X. Zhan, Y. Qie, M. Wang, X. Li, R. Zhao, Selenomethionine: an effective selenium source for sow to improve Se distribution, antioxidant status, and growth performance of pig offspring, Biol. Trace Elem. Res. 142 (3) (2011) 481–491.
[27] J.A. Rooke, I.M. Bland, The acquisition of passive immunity in the new-born piglet, Livest. Prod. Sci. 78 (1) (2002) 13–23.
[28] Z.C. Luo, W.D. Fraser, P. Julien, C.L. Deal, F. Audibert, G.N. Smith, X. Xiong, M. Walker, Tracing the origins of "fetal origins" of adult diseases: programming by oxidative stress? Med. Hypotheses 66 (1) (2006) 38–44.
[29] J.L. McManaman, M.E. Reyland, E.C. Thrower, Secretion and fluid transport mechanisms in the mammary gland: comparisons with the exocrine pancreas and the salivary gland, J. Mammary Gland Biol. Neoplasia 11 (3) (2006) 249–268.
[30] Eur Comm, COUNCIL DIRECTIVE of 23 November 1970 concerning additives in feeding-stuffs (70/524/EEC), OJEC (L 270/1) (1970).
[31] N.F. Suttle, Mineral Nutrition of Livestock, 4th ed., CABI, Wallingford, Oxfordshire, UK, Cambridge, MA, 2010.
[32] EFSA, Scientific opinion on the safety and efficacy of L-selenomethionine as feed additive for all animal species, EFSA J. 11 (5) (2013) 3219.
[33] F.J. Pallarés, M.J. Yaeger, B.H. Janke, G. Fernandez, P.G. Halbur, Vitamin E and selenium concentrations in livers of pigs diagnosed with mulberry heart disease, J. Vet. Diagn. Invest. 14 (5) (2002) 412–414.
[34] Norwegian Meat and Poultry Research Center, Helsetjenesten for svin - Årsrapport 2011, Pig Health Services - Annual Report 2011], (2012) http://www.animalia.no/upload/Filer%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport2011.pdf.
[35] H. Shen, P.R. Thomas, S.M. Ensley, W.I. Kim, A.T. Loynachan, P.G. Halbur, T. Opriessnig, Vitamin E and selenium levels are within normal range in pigs diagnosed with Mulberry Heart Disease and evidence for viral involvement in the syndrome is lacking, Transbound. Emerg. Dis. 58 (6) (2011) 483–491.
[36] M. Oropeza-Moe, H. Wisloff, A. Bernhoft, Selenium deficiency associated porcine and human cardiomyopathies, J. Trace Elem. Med. Biol. 31 (2015) 148–156.
[37] T. Sivertsen, E. Vie, A. Bernhoft, B. Baustad, Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds, Acta Vet. Scand. 49 (2007) 1.
[38] R.J. Henry, N. Chiamori, S.L. Jacobs, M. Segalove, Determination of ceruloplasmin oxidase in serum, Exp. Biol. Med. 104 (4) (1960) 620–624.
[39] M. Falk, A. Bernhoft, T. Framstad, B. Salbu, H. Wisloff, T.M. Kortner, A.B. Kristoffersen, M. Oropeza-Moe, Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs, J. Trace Elem. Med. Biol. (2018).
[40] A. Liba, C. Jones, S. Wilburn, Sensitive, High-throughput Analysis of Lead in Whole Blood Using the Agilent 7500cx ICP-MS With ISIS-DS, Agilent Technologies, Inc., 2015 p. 4 https://www.agilent.com/cs/library/applications/5990-5416EN.pdf.
[42] RStudio Team, R Foundation for Statistical Computing (Ed.), RStudio: Integrated Development for R, RStudio, Inc., Boston, USA, 2016.
[43] W.M. Stanish, N. Taylor, Estimation of the intraclass correlation coefficient for the analysis of covariance model, Am. Stat. 37 (3) (1983) 221–224.
[44] D.T. Nguyen, K. Lee, H. Choi, M.-k. Choi, M.T. Le, N. Song, J.-H. Kim, H.G. Seo, J.-W. Oh, K. Lee, T.-H. Kim, C. Park, The complete swine olfactory subgenome: expansion of the olfactory gene repertoire in the pig genome, BMC Genomics 13 (1) (2012) 584–595.
[45] P.C. Brunjes, S. Feldman, S.K. Osterberg, The pig olfactory brain: a primer, Chem. Senses 41 (5) (2016) 415–425.
[46] M.L. Jankevicius, T.M. Widowski, Does balancing for color affect pigs' preference for different flavored tail-models? Appl. Anim. Behav. Sci. 84 (2) (2003) 159–165.
[47] Z. Taheri, S. Karimi, H. Mehrban, A. Moharrery, Supplementation of different selenium sources during early lactation of native goats and their effects on nutrient digestibility, nitrogen and energy status, J. Appl. Anim. Res. 46 (1) (2016) 64–68.
[48] S.L. Thingnes, E. Hallenstvedt, E. Sandberg, T. Framstad, The effect of different dietary energy levels during rearing and mid-gestation on gilt performance and culling rate, Livest. Sci. 172 (2015) 33–42.
[49] S.L. Thingnes, A.H. Gaustad, N.P. Kjos, E. Sandberg, T. Framstad, The effect of different dietary energy levels during rearing and mid-gestation on sow lifetime performance and longevity, Acta Agric. Scand. A 65 (3-4) (2015) 148–157.
[50] J. Noblet, C. Karege, S. Dubois, J. van Milgen, Metabolic utilization of energy and maintenance requirements in growing pigs: effects of sex and genotype, J. Anim. Sci. 77 (5) (1999) 1208–1216.
[51] R.O. Ball, R.S. Samuel, S. Moehn, Nutrient requirements of prolific sows, Banff pork seminar 2008, Changing the industry with new solutions, Department of Agricultural, Food and Nutritional Science, University of Alberta, Banff, Alberta, Canada, 2008, pp. 223–237.
[52] A.S. Prasad, Zinc: role in immunity, oxidative stress and chronic inflammation, Curr. Opin. Clin. Nutr. Metab. Care 12 (6) (2009) 646–652.
[53] L. Jin, D. Li, G.N. Alesi, J. Fan, H.B. Kang, Z. Lu, T.J. Boggon, P. Jin, H. Yi, E.R. Wright, D. Duong, N.T. Seyfried, R. Egnatchik, R.J. DeBerardinis, K.R. Magliocca, C. He, M.L. Arellano, H.J. Khoury, D.M. Shin, F.R. Khuri, S. Kang, Glutamate dehydrogenase 1 signals through antioxidant glutathione peroxidase 1 to regulate redox homeostasis and tumor growth, Cancer Cell 27 (2) (2015) 257–270.
[54] D.-H. Lee, R. Blomhoff, D.R. Jacobs, Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radical Res. 38 (6) (2004) 535–539.
[55] A. Geetha, P. Saranya, S. Annie Jeyachristy, R. Surendran, A. Sundaram, Relevance of non-ceruloplasmin copper to oxidative stress in patients with hepatocellular carcinoma, Biol. Trace Elem. Res. 130 (3) (2009) 229–240.
[56] I. Yoon, E. McMillan, Comparative effects of organic and inorganic selenium on selenium transfer from sows to nursing pigs, J. Anim. Sci. 84 (7) (2006) 1729–1733.
[57] J.J. Matte, C.L. Girard, Changes of serum and blood volumes during gestation and lactation in multiparous sows, Can. J. Anim. Sci. 76 (1996) 263–266.
[58] M. Kaibara, Y. Marumoto, T. Kobayashi, [Hemodilution and anemia in pregnancy and fetal development], Nihon Sanka Fujinka Gakkai zasshi 36 (10) (1984) 1893–1900.
[59] M. Babicz, R. Cichocki, K. Kasprzak, K. Kropiwiec, K. Szuba, Analysis of relationship between sows colostrum and milk somatic cell count and reproductive performance traits, Ann. Univ. Mariae Curie-Skłodowska. Sectio EE: Zootechnica 3 (29) (2011) 1–13.
[60] J. Csapó, T.G. Martin, Z.S. Csapó-Kiss, Z. Házas, Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days, Int. Dairy J. 6 (8) (1996) 881–902.
[61] S. Zou, D.G. Mclaren, W.L. Hurley, Pig colostrum and milk-composition - Comparisons between Chinese Meishan and United-States breeds, Livest. Prod. Sci. 30 (1-2) (1992) 115–127.
[62] D.C. Mahan, Effect of organic and inorganic selenium sources and levels on sow colostrum and milk selenium content, J. Anim. Sci. 78 (1) (2000) 100–105.
[63] D.C. Mahan, Y.Y. Kim, Effect of inorganic or organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first-parity gilts and their progeny, J. Anim. Sci. 74 (11) (1996) 2711–2718.
[64] B. Pehrson, K. Ortman, N. Madjid, U. Trafikowska, The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of Suckler cows and on the selenium status of their calves, J. Anim. Sci. 77 (12) (1999) 3371–3376.
[65] K. Ortman, B. Pehrson, Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast, J. Anim. Sci. 77 (12) (1999) 3365–3370.
[66] X.A. Zhan, Y.Z. Qie, M. Wang, X. Li, R.Q. Zhao, Selenomethionine: an effective selenium source for sow to improve Se distribution, antioxidant status, and growth performance of pig offspring, Biol. Trace Elem. Res. 142 (3) (2011) 481–491.
[67] H. Hu, M. Wang, X. Zhan, X. Li, R. Zhao, Effect of different selenium sources on productive performance, serum and milk Se concentrations, and antioxidant status of sows, Biol. Trace Elem. Res. 142 (3) (2011) 471–480.
[68] D.C. Mahan, J.C. Peters, Long-term effects of dietary organic and inorganic selenium sources and levels on reproducing sows and their progeny, J. Anim. Sci. 82 (5) (2004) 1343–1358.
[69] S. Fagan, R. Owens, P. Ward, C. Connolly, S. Doyle, R. Murphy, Biochemical comparison of commercial selenium yeast preparations, Biol. Trace Elem. Res. 166 (2) (2015) 245–259.
[70] J.A. Hall, G. Bobe, W.R. Vorachek, C.T. Estill, W.D. Mosher, G.J. Pirelli, M. Gamroth, Effect of supranutritional maternal and colostral selenium supplementation on passive absorption of immunoglobulin G in selenium-replete dairy calves, J. Dairy Sci. 97 (7) (2014) 4379–4391.
[71] C. Kielland, V. Rootwelt, O. Reksen, T. Framstad, The association between immunoglobulin G in sow colostrum and piglet plasma, J. Anim. Sci. 93 (9) (2015) 4453–4462.
[72] K.E. Hill, A.K. Motley, V.P. Winfrey, R.F. Burk, Selenoprotein P is the major selenium transport protein in mouse milk, PLoS One (2014) e103486.
[73] A. Geursen, M.R. Grigor, Serum albumin secretion in rat milk, J. Physiol. 391 (1) (1987) 419–427.
[74] J. Monks, M.C. Neville, Albumin transcytosis across the epithelium of the lactating mouse mammary gland, J. Physiol. 560 (1) (2004) 267–280.
[75] G.E. Olson, V.P. Winfrey, K.E. Hill, R.F. Burk, Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells, J. Biol. Chem. 283 (11) (2008) 6854–6860.
[76] M.J. Rowling, C.M. Kemmis, D.A. Taffany, J. Welsh, Megalin-mediated endocytosis of vitamin D binding protein correlates with 25-hydroxycholecalciferol actions in human mammary cells, J. Nutr. 136 (11) (2006) 2754–2759.
[77] R.R. de la Flor St. Remy, M.L. Fernández Sánchez, J.B. López Sastre, A. Sanz-Medel, Multielemental distribution patterns in premature human milk whey and pre-term formula milk whey by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry with octopole reaction cell, J. Anal. At. Spectrom. 19 (9) (2004) 1104–1110.
[78] F. Fantuz, S. Ferraro, L. Todini, P. Mariani, R. Piloni, E. Salimei, Essential trace elements in milk and blood serum of lactating donkeys as affected by lactation stage and dietary supplementation with trace elements, Animal 7 (11) (2013) 1893–1899.
[79] B. Lönnerdal, Trace element transport in the mammary gland, Annu. Rev. Nutr. 27 (1) (2007) 165–177.
[80] O. Muñiz-Naveiro, R. Domínguez-González, A. Bermejo-Barrera, P. Bermejo-Barrera, J.A. Cocho, J.M. Fraga, Selenium speciation in cow milk obtained after supplementation with different selenium forms to the cow feed using liquid chromatography coupled with hydride generation-atomic fluorescence spectrometry, Talanta 71 (4) (2007) 1587–1593.
[81] R.H. Phipps, A.S. Grandison, A.K. Jones, D.T. Juniper, E. Ramos-Morales, G. Bertin,

Selenium supplementation of lactating dairy cows: effects on milk production and total selenium content and speciation in blood, milk and cheese, Animal 2 (11) (2008) 1610–1618.
[82] A.T. Mudd, L.S. Alexander, S.K. Johnson, C.M. Getty, O.V. Malysheva, M.A. Caudill, R.N. Dilger, Perinatal dietary choline deficiency in sows influences concentrations of choline metabolites, fatty acids, and amino acids in milk throughout lactation, J. Nutr. 146 (11) (2016) 2216–2223.
[83] K.E. Hill, Y. Xia, B. Åkesson, M.E. Boeglin, R.F. Burk, Selenoprotein P concentration in plasma is an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects, J. Nutr. 126 (1) (1996) 138–145.
[84] P.D. Whanger, J.A. Butler, J.T. Deagen, Determination of the distribution of selenium between selenoprotein P, glutathione peroxidase and albumin in plasma, 75, Annual meeting of the Federation of American Societies for Experimental Biology (FASEB), FASEB Journal (Federation of American Societies for Experimental Biology) (1991).
[85] J.A. Milner, L. Sherman, M.F. Picciano, Distribution of selenium in human milk, Am. J. Clin. Nutr. 45 (3) (1987) 617–624.
[86] N. Avissar, J. Whitin, P. Allen, I. Palmer, H. Cohen, Antihuman plasma glutathione peroxidase antibodies: immunologic investigations to determine plasma glutathione peroxidase protein and selenium content in plasma, Blood 73 (1) (1989) 318–323.
[87] B. Debski, M.F. Picciano, J.A. Milner, Selenium content and distribution of human, cow and goat milk, J. Nutr. 117 (6) (1987) 1091–1097.
[88] G.E. Olson, J.C. Whitin, K.E. Hill, V.P. Winfrey, A.K. Motley, L.M. Austin, J. Deal, H.J. Cohen, R.F. Burk, Extracellular glutathione peroxidase (Gpx3) binds specifically to basement membranes of mouse renal cortex tubule cells, Am. J. Physiol. Renal Physiol. 298 (5) (2010) F1244–F1253.
[89] K. Bierla, J. Szpunar, R. Lobinski, Specific determination of selenoaminoacids in whole milk by 2D size-exclusion-ion-paring reversed phase high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP MS), Anal. Chim. Acta 624 (2) (2008) 195–202.

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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)$^{b^}$	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 ($P < 0.0083$). ^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-]				
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). ^P = 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

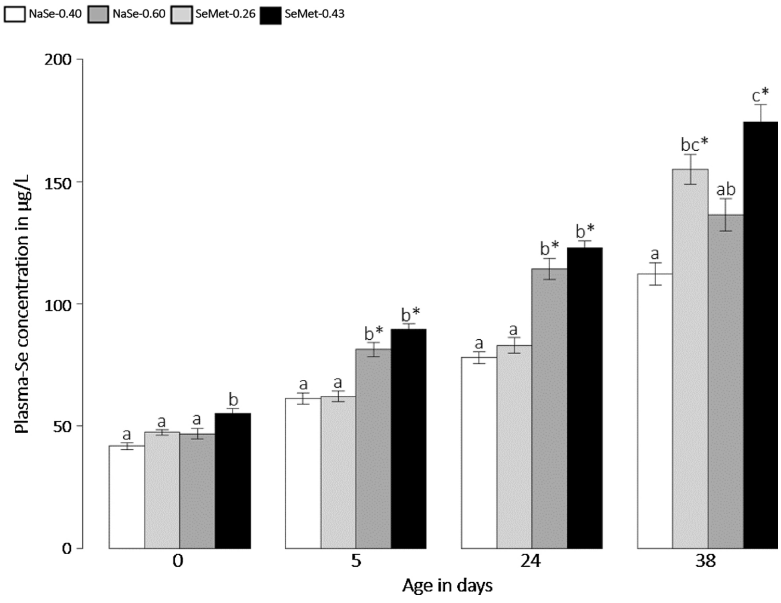

Fig. 1. Total selenium concentrations in plasma of piglets originated from sows fed with sodium selenite (NaSe-0.40, NaSe-0.60) and L-selenomethionine (SeMet-0.26, SeMet-0.43) enriched diets sampled at birth and 5, 24 and 38 days of age (n = 15/15/6/9, 15/15/6/8, 15/15/7/9, 12/15/7/11 for each diet and 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.

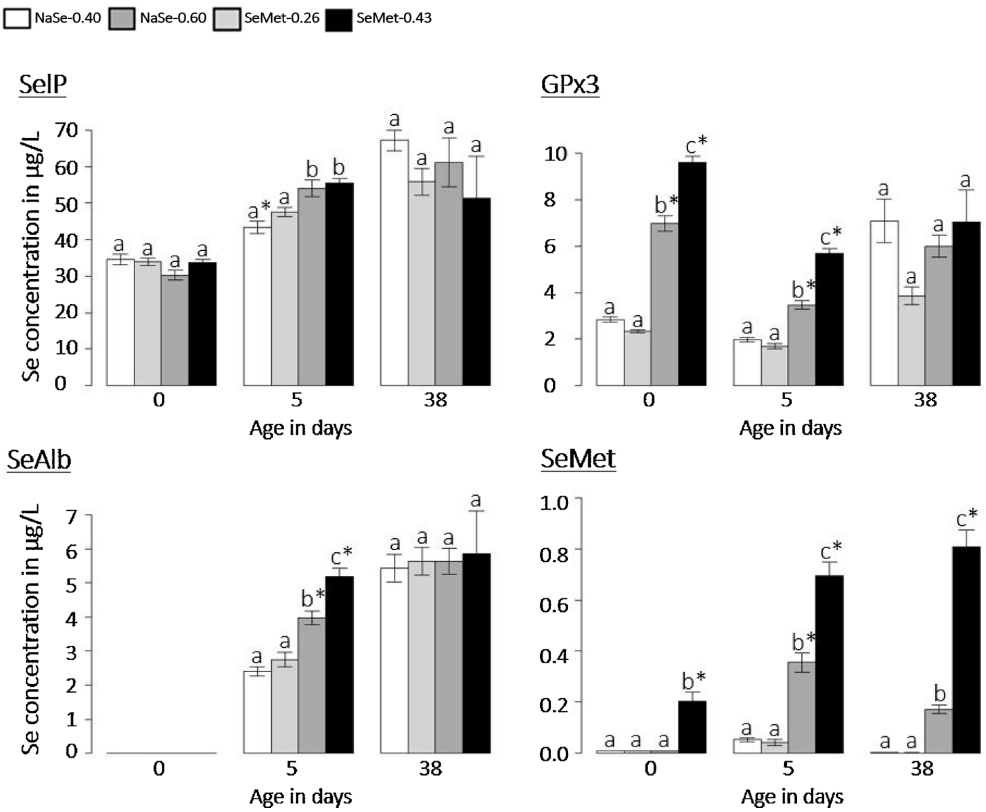

Fig. 2. Distribution of Se biomoecules in plasma of piglets originated from sows fed with sodium selenite (NaSe-0.40, NaSe-0.60) and L-selenomethionine (SeMet-0.26, SeMet-0.43) diets at 0, 5 and 38 days of age (n = 15/15/9, 5/15/9, 15/15/9, 15/15/11 for each diet and each time point). Bar plots represent 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.

References

[1] T. Norsvin, Avlsmål Duroc [Breeding goals Duroc], (2012) (Accessed 23.05.2017 2017).
[2] Topigs Norsvin, Norsvin Landrace, The Insider, Topigs Norsvin, http://topigsnorsvin.ca/wp-content/uploads/2015/04/Topigs-Norsvin-Insider-1412.docx.pdf, 2014, pp. 4–5.
[3] Topigs Norsvin, Topigs Norsvin LZ 70, The Insider, Topigs Norsvin, http://topigsnorsvin.ca/wp-content/uploads/2015/04/Autumn-Topigs-Norsvin-Insider-1409.pdf, 2014, p. 5.
[4] C. Alonso-Alvarez, S. Bertrand, B. Faivre, G. Sorci, Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches, Funct. Ecol. 21 (5) (2007) 873–879.
[5] H.M. Brown-Borg, S.G. Rakoczy, Growth hormone administration to long-living dwarf mice alters multiple components of the antioxidative defense system, Mech. Ageing Dev. 124 (10–12) (2003) 1013–1024.
[6] T.K. Nielsen, C. Wolstrup, A.L. Schirmer, P.T. Jensen, Mulberry Heart Disease in young pigs without vitamin E and selenium deficiency, Vet. Rec. 124 (20) (1989) 535–537.
[7] P.A. Dennery, Role of redox in fetal development and neonatal diseases, Antioxid. Redox Signal. 6 (1) (2004) 147–153.
[8] Z.C. Luo, W.D. Fraser, P. Julien, C.L. Deal, F. Audibert, G.N. Smith, X. Xiong, M. Walker, Tracing the origins of "fetal origins" of adult diseases: programming by oxidative stress? Med. Hypotheses 66 (1) (2006) 38–44.
[9] P.F. Surai, V.I. Fisinin, Selenium in sow nutrition, Anim. Feed Sci.Techn. 211 (2016) 18–30.
[10] J.M. Finch, R.J. Turner, Effects of selenium and vitamin E on the immune responses of domestic animals, Res. Vet. Sci. 60 (2) (1996) 97–106.
[11] M. Birringer, S. Pilawa, L. Flohe, Trends in selenium biochemistry, Nat. Prod. Rep. 19 (6) (2002) 693–718.
[12] L.V. Papp, J. Lu, A. Holmgren, K.K. Khanna, From selenium to selenoproteins: synthesis, identity, and their role in human health, Antioxid. Redox Signal. 9 (7) (2007) 775–806.
[13] V. Pagmantidis, C. Meplan, E.M. van Schothorst, J. Keijer, J.E. Hesketh, Supplementation of healthy volunteers with nutritionally relevant amounts of selenium increases the expression of lymphocyte protein biosynthesis genes, Am. J. Clin. Nutr. 87 (1) (2008) 181–189.
[14] H. Steinbrenner, H. Sies, Protection against reactive oxygen species by selenoproteins, Biochim. Biophys. Acta 1790 (11) (2009) 1478–1485.
[15] J. Pinsent, The need for selenite and molybdate in the formation of formic dehydrogenase by members of the Coli-aerogenes group of bacteria, Biochem. J. 57 (1) (1954) 10–16.
[16] K. Schwarz, C.M. Foltz, Selenium as an integral part of Factor-3 against dietary necrotic liver degeneration, J. Am. Chem. Soc. 79 (12) (1957) 3292–3293.
[17] A.C. Pappas, E. Zoidis, P.F. Surai, G. Zervas, Selenoproteins and maternal nutrition, Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 151 (4) (2008) 361–372.
[18] M. Falk, A. Bernhoft, T. Framstad, B. Salbu, H. Wisloff, T.M. Kortner, A.B. Kristoffersen, M. Oropeza-Moe, Effects of dietary sodium selenite and organic selenium sources on immune and inflammatory responses and selenium deposition in growing pigs, J. Trace Elem. Med. Biol. 50 (2018) 527–536.
[19] M. Falk, P. Lebed, A. Bernhoft, T. Framstad, A.B. Kristoffersen, B. Salbu, M. Oropeza-Moe, Effects of sodium selenite and L-selenomethionine on feed intake, clinically relevant blood parameters and selenium species in plasma, colostrum and milk from high-yielding sows, J. Trace Elem. Med. Biol. 52 (2019) 176–185.
[20] D.B. Dalto, I. Audet, J. Lapointe, J.J. Matte, The importance of pyridoxine for the impact of the dietary selenium sources on redox balance, embryo development, and reproductive performance in gilts, J. Trace Elem. Med. Biol. 34 (2016) 79–89.
[21] L. Guan, B. Han, Z. Li, F. Hua, F. Huang, W. Wei, Y. Yang, C. Xu, Sodium selenite induces apoptosis by ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction in human acute promyelocytic leukemia NB4 cells, Apoptosis 14 (2) (2009) 218–225.
[22] Y. Seko, Y. Saito, J. Kitahara, N. Imura, Active oxygen generation by the reaction of selenite with reduced glutathione in vitro, in: A. Wendel (Ed.), Selenium in Biology and Medicine, Springer Berlin Heidelberg, Berlin, Heidelberg, 1989, pp. 70–73.
[23] H. Hu, M. Wang, X. Zhan, X. Li, R. Zhao, Effect of different selenium sources on productive performance, serum and milk Se concentrations, and antioxidant status of sows, Biol. Trace Elem. Res. 142 (3) (2011) 471–480.
[24] D. Solà-Oriol, J. Gasa, Feeding strategies in pig production: sows and their piglets, Anim Feed Sci Tech 233 (2017) 34–52.
[25] C. Lauridsen, H. Engel, S.K. Jensen, A.M. Craig, M.G. Traber, Lactating sows and suckling piglets preferentially incorporate *RRR*- over *all-rac-α*-tocopherol into milk, plasma and tissues, J. Nutr. 132 (6) (2002) 1258–1264.
[26] P.A. Morrissey, T.R. Hill, Fat-soluble vitamins and vitamin C in milk and milk products, in: P. McSweeney, P.F. Fox (Eds.), Advanced Dairy Chemistry, Vol. 3 Springer New York, New York, NY, 2009, pp. 527–589 Lactose, Water, Salts and Minor Constituents.
[27] V.M. Labunskyy, D.L. Hatfield, V.N. Gladyshev, Selenoproteins: molecular pathways and physiological roles, Physiol. Rev. 94 (3) (2014) 739–777.
[28] N. Hidiroglou, N. Cave, A.S. Atwall, E.R. Farnworth, L.R. McDowell, Comparative vitamin E requirements and metabolism in livestock, Ann. Rech. Vet. 23 (4) (1992) 337–359.
[29] L.J. Machlin, A. Bendich, Free radical tissue damage: protective role of antioxidant nutrients, FASEB J. 1 (6) (1987) 441–445.
[30] B. Halliwell, J.M. Gutteridge, The antioxidants of human extracellular fluids, Arch. Biochem. Biophys. 280 (1) (1990) 1–8.
[31] J. Teige, S. Tollersrud, A. Lund, H.J. Larsen, Swine dysentery: the influence of dietary vitamin E and selenium on the clinical and pathological effects of Treponema hyodysenteriae infection in pigs, Res. Vet. Sci. 32 (1) (1982) 95–100.
[32] H. Wuryastuti, H.D. Stowe, R.W. Bull, E.R. Miller, Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum, and milk leukocytes of sows, J. Anim. Sci. 71 (9) (1993) 2464–2472.
[33] The Council of the European Communities, COUNCIL DIRECTIVE of 23 November 1970 concerning additives in feeding-stuffs (70/524/EEC), OJEC (1970) (L 270/1).
[34] Eur Comm, COMMISSION IMPLEMENTING REGULATION (EU) No 445/2013 of 14 May 2013 concerning the authorisation of hydroxy-analogue of selenomethionine as a feed additive for all animal species, Off. J. Eur. Union L 130 (2013) 21–23.
[35] R.J. Henry, N. Chiamori, S.L. Jacobs, M. Segalove, Determination of ceruloplasmin oxidase in serum, Exp. Biol. Med. 104 (4) (1960) 620–624.
[36] H. Fuhrmann, H.P. Sallmann, E. Thesing, [Effects of vitamins A and E on the antioxidative metabolism of weaning pigs given dietary fats of different qualities], Dtsch Tierarztl Wochenschr 104 (9) (1997) 387–391.
[37] A. Brandt-Kjelsen, E. Govasmark, A. Haug, B. Salbu, Turnover of Se in adequately fed chickens using Se-75 as a tracer, J. Anim. Physiol. Anim. Nutr. (Berl) 98 (3) (2014) 547–558.
[38] Y.-F. Li, L. Hu, B. Li, X. Huang, E.H. Larsen, Y. Gao, Z. Chai, C. Chen, Full quantification of selenium species by RP and AF-ICP-qMS with on-line isotope dilution in serum samples from mercury-exposed people supplemented with selenium-enriched yeast, J. Anal. At. Spectrom. 26 (1) (2011) 224–229.
[39] S. Letsiou, Y. Lu, T. Nomikos, S. Antonopoulou, D. Panagiotakos, C. Pitsavos, C. Stefanadis, S.A. Pergantis, High-throughput quantification of selenium in individual serum proteins from a healthy human population using HPLC on-line with isotope dilution inductively coupled plasma-MS, Proteomics 10 (19) (2010) 3447–3457.
[40] RStudio Team, R Foundation for Statistical Computing (Ed.), RStudio: Integrated Development for R. RStudio, Inc., Boston, USA, 2016.
[41] N. Devillers, J. Le Dividich, A. Prunier, Influence of colostrum intake on piglet survival and immunity, Animal 5 (10) (2011) 1605–1612.
[42] Ingris, Årsstatistikk 2015 (Annual statitics 2015), http://www.animalia.no/upload/Ingris%20web/Ingris_A%cc%8arsstatistikk_2015.pdf, 2016.
[43] X.A. Zhan, Y.Z. Qie, M. Wang, X. Li, R.Q. Zhao, Selenomethionine: an effective selenium source for sow to improve Se distribution, antioxidant status, and growth performance of pig offspring, Biol. Trace Elem. Res. 142 (3) (2011) 481–491.
[44] V. Rootwelt, O. Reksen, W. Farstad, T. Framstad, Blood variables and body weight gain on the first day of life in crossbred pigs and importance for survival, J. Anim. Sci. 90 (4) (2012) 1134–1141.
[45] D.C. Mahan, Y.Y. Kim, Effect of inorganic or organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first-parity gilts and their progeny, J. Anim. Sci. 74 (11) (1996) 2711–2718.
[46] D.C. Mahan, J.C. Peters, Long-term effects of dietary organic and inorganic selenium sources and levels on reproducing sows and their progeny, J. Anim. Sci. 82 (5) (2004) 1343–1358.
[47] H. Quesnel, A. Renaudin, N. Le Floc'h, C. Jondreville, M.C. Pere, J.A. Taylor-Pickard, J. Le Dividich, Effect of organic and inorganic selenium sources in sow diets on colostrum production and piglet response to a poor sanitary environment after weaning, Animal 2 (6) (2008) 859–866.
[48] M.P. Rayman, The use of high-selenium yeast to raise selenium status: how does it measure up? Br. J. Nutr. 92 (4) (2004) 557–573.
[49] P.C. Uden, H. Totoe Boakye, C. Kahakachchi, R. Hafezi, P. Nolibos, E. Block, S. Johnson, J.F. Tyson, Element selective characterization of stability and reactivity of selenium species in selenized yeast, J. Anal. At. Spectrom. 19 (1) (2004) 65–73.
[50] E.H. Larsen, J. Sloth, M. Hansen, S. Moesgaard, Selenium speciation and isotope composition in 77Se-enriched yeast using gradient elution HPLC separation and ICP-dynamic reaction cell-MS, J. Anal. At. Spectrom. 18 (4) (2003) 310–316.
[51] M.B. Jornstedt, S. Kumar, A. Holmgren, Selenite and Selenodiglutathione: Reactions With Thioredoxin Systems, Methods in Enzymology, Academic Press, 1995, pp. 209–219.
[52] A. Holmgren, Selenoproteins of the thioredoxin system, in: D.L. Hatfield (Ed.), Selenium: Its Molecular Biology and Role in Human Health, Springer US, Boston, MA, 2001, pp. 179–188.
[53] A. Sengupta, U.F. Lichti, B.A. Carlson, A.O. Ryscavage, V.N. Gladyshev, S.H. Yuspa, D.L. Hatfield, Selenoproteins are essential for proper keratinocyte function and skin development, PLoS One 5 (8) (2010) e12249.
[54] M.A. Foroughi, H. Dehghani, N. Mahdavi-Shahri, M.R. Bassami, Sodium selenite increases the transcript levels of iodothyronine deiodinases I and II in ovine and bovine fetal thyrocytes in vitro, J. Trace Elem. Med. Biol. 27 (3) (2013) 213–220.
[55] F.T. Awadeh, R.L. Kincaid, K.A. Johnson, Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves, J. Anim. Sci. 76 (4) (1998) 1204–1215.
[56] J.R. Arthur, G.J. Beckett, Thyroid function, Br. Med. Bull. 55 (3) (1999) 658–668.
[57] H. Zhong, H. Li, G. Liu, H. Wan, Y. Mercier, X. Zhang, Y. Lin, L. Che, S. Xu, L. Tang,

G. Tian, D. Chen, D. Wu, Z. Fang, Increased maternal consumption of methionine as its hydroxyl analog promoted neonatal intestinal growth without compromising maternal energy homeostasis, J. Anim. Sci. Biotechnol. 7 (1) (2016) 46.
[58] S. Altmann, E. Murani, M. Schwerin, C.C. Metges, K. Wimmers, S. Ponsuksili, Maternal dietary protein restriction and excess affects offspring gene expression and methylation of non-SMC subunits of condensin I in liver and skeletal muscle, Epigenetics 7 (3) (2012) 239–252.
[59] Y. Chen, D. Li, Z. Dai, X. Piao, Z. Wu, B. Wang, Y. Zhu, Z. Zeng, L-Methionine supplementation maintains the integrity and barrier function of the small-intestinal mucosa in post-weaning piglets, Amino Acids 46 (4) (2014) 1131–1142.
[60] C. Bauchart-Thevret, B. Stoll, S. Chacko, D.G. Burrin, Sulfur amino acid deficiency upregulates intestinal methionine cycle activity and suppresses epithelial growth in neonatal pigs, Am. J. Physiol. Endocrinol. Metab. 296 (6) (2009) E1239–E1250.
[61] D.G. Dale, M.A. Macdonald, J.E. Moxley, Hemoglobin levels of piglets at birth and at 21 days and their relation to weight at 154 days of age, Can. J. Comp. Med. Vet. Sci. 25 (8) (1961) 193–197.
[62] C. Liao, R.C. Hardison, M.J. Kennett, B.A. Carlson, R.F. Paulson, K.S. Prabhu, Selenoproteins regulate stress erythroid progenitors and spleen microenvironment during stress erythropoiesis, Blood 131 (23) (2018) 2568–2580.
[63] C. Liao, B.A. Carlson, R.F. Paulson, K.S. Prabhu, The intricate role of selenium and selenoproteins in erythropoiesis, Free Radic. Biol. Med. 127 (2018) 165–171.
[64] F. Simard, F. Guay, C.L. Girard, A. Giguere, J.P. Laforest, J.J. Matte, Effects of concentrations of cyanocobalamin in the gestation diet on some criteria of vitamin B12 metabolism in first-parity sows, J. Anim. Sci. 85 (12) (2007) 3294–3302.
[65] D.M. Ballance, J.D. House, Nonruminant nutrition: amino acids and dietary restrictions, J. Anim. Sci./J. Diary Sci. 83/88 (Suppl.1/Suppl. 1) (2005) 160.
[66] P.H. Chase, J.J. Volpe, L. Laster, Transsulfuration in mammals: fetal and early development of methionine-activating enzyme and its relation to hormonal influences, J. Clin. Invest. 47 (9) (1968) 2099–2108.
[67] G. Gaull, J.A. Sturman, N.C. Raiha, Development of mammalian sulfur metabolism: absence of cystathionase in human fetal tissues, Pediatr. Res. 6 (6) (1972) 538–547.
[68] J.A. Sturman, G. Gaull, N.C. Raiha, Absence of cystathionase in human fetal liver: is cystine essential? Science 169 (3940) (1970) 74–76.
[69] C.L. White, W.G. Hoekstra, The metabolism of selenite and selenomethionine in mouse fibroblasts grown in tissue culture, Biol. Trace Elem. Res. 1 (3) (1979) 243–257.
[70] G. Alfthan, A. Aro, H. Arvilommi, J.K. Huttunen, Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate, Am. J. Clin. Nutr. 53 (1) (1991) 120–125.
[71] M.A. Beilstein, P.D. Whanger, Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine, J. Nutr. (1986) 1711–1719.
[72] N. Kaushal, S. Hegde, J. Lumadue, R.F. Paulson, K.S. Prabhu, The regulation of erythropoiesis by selenium in mice, Antioxid. Redox Signal. 14 (8) (2011) 1403–1412.
[73] P. Porayette, R.F. Paulson, BMP4/Smad5 dependent stress erythropoiesis is required for the expansion of erythroid progenitors during fetal development, Dev. Biol. (Basel) 317 (1) (2008) 24–35.
[74] A. Bogdanova, A. Makhro, J. Wang, P. Lipp, L. Kaestner, Calcium in red blood cells-a perilous balance, Int. J. Mol. Sci. 14 (5) (2013) 9848–9872.
[75] N. Petit, A. Lescure, M. Rederstorff, A. Krol, B. Moghadaszadeh, U.M. Wewer, P. Guicheney, Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern, Hum. Mol. Gen. 12 (9) (2003) 1045–1053.
[76] A.C. Uğuz, M. Naziroğlu, J. Espino, I. Bejarano, D. González, A.B. Rodríguez, J.A. Pariente, Selenium modulates oxidative stress-induced cell apoptosis in human myeloid HL-60 cells through regulation of calcium release and caspase-3 and -9 activities, J. Membr. Biol. 232 (1-3) (2009) 15–23.
[77] J. Liu, S. Rozovsky, Membrane-bound selenoproteins, Antioxid. Redox Signal. 23 (10) (2015) 795–813.
[78] A. Kugelman, H.A. Choy, R. Liu, M.M. Shi, E. Gozal, H.J. Forman, Gamma-Glutamyl transpeptidase is increased by oxidative stress in rat alveolar L2 epithelial cells, Am. J. Respir. Cell Mol. Biol. 11 (5) (1994) 586–592.
[79] E.A. Golenkina, G.M. Viryasova, S.I. Galkina, T.V. Gaponova, G.F. Sud'ina, A.V. Sokolov, Fine regulation of neutrophil oxidative status and apoptosis by ceruloplasmin and its derivatives, Cells 7 (1) (2018) 8.
[80] D.-H. Lee, R. Blomhoff, D.R. Jacobs, Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic. Res. Commun. 38 (6) (2004) 535–539.
[81] M.W. Lieberman, A.L. Wiseman, Z.Z. Shi, B.Z. Carter, R. Barrios, C.N. Ou, P. Chévez-Barrios, Y. Wang, G.M. Habib, J.C. Goodman, S.L. Huang, R.M. Lebovitz, M.M. Matzuk, Growth retardation and cysteine deficiency in gamma-glutamyl transpeptidase-deficient mice, Proc. Natl. Acad. Sci. U.S.A. 93 (15) (1996) 7923–7926.
[82] T. Schnelldorfer, S. Gansauge, F. Gansauge, S. Schlosser, H.G. Beger, A.K. Nussler, Glutathione depletion causes cell growth inhibition and enhanced apoptosis in pancreatic cancer cells, Cancer 89 (7) (2000) 1440–1447.
[83] R.H. Burdon, D. Alliangana, V. Gill, Endogenously generated active oxygen species and cellular glutathione levels in relation to BHK-21 cell proliferation, Free Radic. Res. 21 (3) (1994) 121–133.
[84] R.M. Day, Y.J. Suzuki, B.L. Fanburg, Regulation of glutathione by oxidative stress in bovine pulmonary artery endothelial cells, Antioxid. Redox Signal. 5 (6) (2003) 699–704.
[85] O.W. Griffith, Biologic and pharmacologic regulation of mammalian glutathione synthesis, Free Radical Biol. Med. 27 (9) (1999) 922–935.
[86] Z. Luo, W. Zhu, Q. Guo, W. Luo, J. Zhang, W. Xu, J. Xu, Weaning induced hepatic oxidative stress, apoptosis, and aminotransferases through MAPK signaling pathways in piglets, Oxid. Med. Cell. Longev. 2016 (2016) 10.
[87] H.M. Shen, C.F. Yang, C.N. Ong, Sodium selenite-induced oxidative stress and apoptosis in human hepatoma HepG2 cells, Int. J. Cancer 81 (5) (1999) 820–828.
[88] M.S. Stewart, J.E. Spallholz, K.H. Neldner, B.C. Pence, Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis, Free Radic. Biol. Med. 26 (1–2) (1999) 42–48.
[89] D.C. Mahan, Assessment of the influence of dietary vitamin E on sows and offspring in 3 parties - reproductive performance, tissue tocopherol, and effects on progeny, J. Anim. Sci. 69 (7) (1991) 2904–2917.
[90] D.C. Mahan, Effects of dietary vitamin E on sow reproductive performance over a five-parity period, J. Anim. Sci. 72 (11) (1994) 2870–2879.
[91] A. Malm, W.G. Pond, E.F. Walker Jr., M. Homan, A. Aydin, D. Kirtland, Effect of polyunsaturated fatty acids and vitamin E level of the sow gestation diet on reproductive performance and on level of alpha tocopherol in colostrum, milk and dam and progeny blood serum, J. Anim. Sci. 42 (2) (1976) 393–399.
[92] I. Chambers, J. Frampton, P. Goldfarb, N. Affara, W. McBain, P.R. Harrison, The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the' termination' codon, TGA, EMBO J. 5 (6) (1986) 1221–1227.
[93] F. Wang, T. Wang, J. Lai, M. Li, C. Zou, Vitamin E inhibits hemolysis induced by hemin as a membrane stabilizer, Biochem. Pharmacol. 71 (6) (2006) 799–805.
[94] B. Gradinski-Vrbanac, Z. Stojević, S. Milinković-Tur, T. Balenović, J. Pirsljin, In vitro susceptibility of duck, chicken, and pig erythrocyte lipids to peroxidation, Vet. Med. 47 (10–11) (2002) 303–308.
[95] S. Flory, M. Birringer, J. Frank, Bioavailability and metabolism of vitamin E, in: P. Weber, M. Birringer, J.B. Blumberg, M. Eggersdorfer, J. Frank (Eds.), Vitamin E in Human Health, Springer International Publishing, Cham, 2019, pp. 31–41.
[96] A. Stocker, Molecular mechanisms of vitamin E transport, Ann. N. Y. Acad. Sci. 1031 (1) (2004) 44–59.
[97] Y. Sun, A. Ma, Y. Li, X. Han, Q. Wang, H. Liang, Vitamin E supplementation protects erythrocyte membranes from oxidative stress in healthy Chinese middle-aged and elderly people, Nutr. Res. 32 (5) (2012) 328–334.
[98] Norwegian Meat and Poultry Research Center, Helsetjenesten for Svin - Årsrapport 2011 [Pig Health Services - Annual Report 2011], (2012) http://www.animalia.no/upload/FIler%20til%20nedlasting/HTsvin/Publikasjoner/%c3%85rsrapport2011.pdf.
[99] J. Shurson, A. Hanson, L. Johnston, S. Baidoo, J. Torrison, C. Chen, Vitamin E and Selenium Status of Pigs Fed DDGS Diets and Relationship to Mulberry Heart Disease, Agricultural utilization research institute, 2013 p. 22 http://www.auri.org/assets/2013/01/Vitamin-E-and-selenium-status-of-pigs-fed-DDGS-diets-and-relationship-to-Mulberry-Heart-Disease1.pdf.
[100] C. Lauridsen, S.K. Jensen, Influence of supplementation of all-rac-α-tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and immune responses of piglets, J. Anim. Sci. 83 (6) (2005) 1274–1286.
[101] T. Sivertsen, E. Vie, A. Bernhoft, B. Baustad, Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds, Acta Vet. Scand. 49 (2007) 1.
[102] F.J. Pallarés, M.J. Yaeger, B.H. Janke, G. Fernandez, P.G. Halbur, Vitamin E and selenium concentrations in livers of pigs diagnosed with mulberry heart disease, J. Vet. Diagn. Invest. 14 (5) (2002) 412–414.
[103] S. Done, S.M. Williamson, B.W. Strugnell, Nervous and locomotor systems, in: J.J. Zimmerman, L.A. Karriker, A. Ramirez, K.J. Schwartz, G.W. Stevenson (Eds.), Diseases of Swine. 10th ed., John Wiley and Sons Ltd, UK, 2012, pp. 294–328.
[104] B.J. Cooper, B.A. Valentine, Muscle and tendon, in: G. Maxie (Ed.), Jubb, Kennedy & Palmer'S Pathology of Domestic Animals W.B. Saunders, 2016, pp. 164–249.
[105] W.F. Robinson, N.A. Robinson, Cardiovascular system, in: M.G. Maxie (Ed.), Jubb, Kennedy, and Palmer'S Pathology of Domestic Animals, Elsevier, Inc., 2015, pp. 1–101.
[106] I. Yoon, E. McMillan, Comparative effects of organic and inorganic selenium on selenium transfer from sows to nursing pigs, J. Anim. Sci. 84 (7) (2006) 1729–1733.
[107] A.M. Finch, L.G. Yang, M.O. Nwagwu, K.R. Page, H.J. McArdle, C.J. Ashworth, Placental transport of leucine in a porcine model of low birth weight, Reproduction 128 (2) (2004) 229.
[108] D.C. Mahan, A.L. Moxon, M. Hubbard, Efficacy of inorganic selenium supplementation to sow diets on resulting carry-over to their progeny, J. Anim. Sci. 45 (4) (1977) 738–746.
[109] M.E. Fortier, I. Audet, A. Giguere, J.P. Laforest, J.F. Bilodeau, H. Quesnel, J.J. Matte, Effect of dietary organic and inorganic selenium on antioxidant status, embryo development, and reproductive performance in hyperovulatory first-parity gilts, J. Anim. Sci. 90 (1) (2012) 231–240.
[110] D.C. Mahan, Effect of organic and inorganic selenium sources and levels on sow colostrum and milk selenium content, J. Anim. Sci. 78 (1) (2000) 100–105.
[111] S. Fagan, R. Owens, P. Ward, C. Connolly, S. Doyle, R. Murphy, Biochemical comparison of commercial selenium yeast preparations, Biol. Trace Elem. Res. 166 (2) (2015) 245–259.
[112] J. Far, H. Preud'homme, R. Lobinski, Detection and identification of hydrophilic selenium compounds in selenium-rich yeast by size exclusion–microbore normal-phase HPLC with the on-line ICP–MS and electrospray Q-TOF-MS detection, Anal. Chim. Acta 657 (2) (2010) 175–190.
[113] A.P. Kipp, D. Strohm, R. Brigelius-Flohé, L. Schomburg, A. Bechthold, E. Leschik-Bonnet, H. Heseker, Revised reference values for selenium intake, J. Trace Elem. Med. Biol. 32 (2015) 195–199.
[114] R.F. Burk, G.E. Olson, K.E. Hill, V.P. Winfrey, A.K. Motley, S. Kurokawa, Maternal-fetal transfer of selenium in the mouse, FASEB J. 27 (8) (2013) 3249–3256.
[115] N. Solanky, A. Requena Jimenez, S.W. D'Souza, C.P. Sibley, J.D. Glazier,

Expression of folate transporters in human placenta and implications for homocysteine metabolism, Placenta 31 (2) (2010) 134–143.
[116] P. Patel, M. Vatish, J. Heptinstall, R. Wang, R.J. Carson, The endogenous production of hydrogen sulphide in intrauterine tissues, Reprod. Biol. Endocrinol. 7 (1) (2009) 10.
[117] D. Katzer, A. Mueller, L. Welzing, H. Reutter, J. Reinsberg, P. Bartmann, S. Bagci, Antioxidative status and oxidative stress in the fetal circulation at birth: the effects of time of delivery and presence of labor, Early Hum. Dev. 91 (2) (2015) 119–124.
[118] D.C. Mahan, A.L. Moxon, J.H. Cline, Efficacy of supplemental selenium in reproductive diets on sow and progeny serum and tissue selenium values, J. Anim. Sci. 40 (4) (1975) 624–631.
[119] K.E. Hill, A.K. Motley, V.P. Winfrey, R.F. Burk, Selenoprotein P is the major selenium transport protein in mouse milk, PLoS One (2014) e103486.
[120] K.T. Suzuki, Y. Ogra, Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se, Food Addit. Contam. 19 (10) (2002) 974–983.
[121] G.N. Thompson, D. Halliday, Protein turnover in pregnancy, Eur. J. Clin. Nutr. 46 (6) (1992) 411–417.
[122] V. Bekenev, A. Garcia, V. Hasnulin, Adaptation of piglets using different methods of stress prevention, Animals 5 (2) (2015) 349–360.
[123] A.J. Moeser, C.S. Pohl, M. Rajput, Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs, Anim. Nutr. Feed Technol. 3 (4) (2017) 313–321.
[124] M. Svoboda, R. Ficek, J. Drabek, Efficacy of organic selenium from se-enriched yeast on selenium transfer from sows to piglets, Acta Vet Brno 77 (4) (2008) 515–521.
[125] Y.L. Ma, M.D. Lindemann, J.L. Pierce, J.M. Unrine, G.L. Cromwell, Effect of inorganic or organic selenium supplementation on reproductive performance and tissue trace mineral concentrations in gravid first-parity gilts, fetuses, and nursing piglets, J. Anim. Sci. 92 (12) (2014) 5540–5550.
[126] G.N. Schrauzer, Nutritional selenium supplements: product types, quality, and safety, J. Am. Coll. Nutr. 20 (1) (2001) 1–4.
[127] D. Behne, H. Weiler, A. Kyriakopoulos, Effects of selenium deficiency on testicular morphology and function in rats, J. Reprod. Fertil. 106 (2) (1996) 291–297.
[128] G.E. Olson, V.P. Winfrey, K.E. Hill, R.F. Burk, Sequential development of flagellar defects in spermatids and epididymal spermatozoa of selenium-deficient rats, Reproduction 127 (3) (2004) 335–342.
[129] P. Kaur, M.P. Bansal, Effect of oxidative stress on the spermatogenic process and hsp70 expressions in mice testes, Indian J. Biochem. Biophys. 40 (4) (2003) 246–251.
[130] R. Kaur, V.R. Parshad, Effects of dietary selenium on differentiation, morphology and functions of spermatozoa of the house rat, Ratuus rattus L, Mutat Res. Fund Mol. Mech. Mut. 309 (1) (1994) 29–35.
[131] N. Kaushal, M.P. Bansal, Selenium variation induced oxidative stress regulates p53 dependent germ cell apoptosis: plausible involvement of HSP70-2, Eur. J. Nutr. 48 (4) (2009) 221–227.
[132] Eur Comm, COUNCIL DIRECTIVE of 23 November 1970 concerning additives in feeding-stuffs (70/524/EEC), OJEC (1970) (L 270/1).
[133] J.J. Dougherty, W.G. Hoekstra, Stimulation of lipid peroxidation in vivo by injected selenite and lack of stimulation by selenate, Proc. Soc. Exp. Biol. Med. 169 (2) (1982) 209–215.
[134] G.N. Schrauzer, Selenomethionine: a review of its nutritional significance, metabolism and toxicity, J. Nutr. 130 (7) (2000) 1653–1656.
[135] M.J. Loudenslager, P.K. Ku, P.A. Whetter, D.E. Ullrey, C.K. Whitehair, H.D. Stowe, E.R. Miller, Importance of diet of dam and colostrum to the biological antioxidant status and parenteral iron tolerance of the pig, J. Anim. Sci. 63 (6) (1986) 1905–1914.
[136] J. Håkansson, J. Hakkarainen, N. Lundeheim, Variation in vitamin E, glutathione peroxidase and retinol concentrations in blood plasma of primiparous sows and their piglets, and in vitamin E, selenium and retinol contents in sows' milk, Acta Agric. Scand. A 51 (4) (2001) 224–234.
[137] C.E. Thorn, Hematology of the pig, in: D.J. Weiss, K.J. Wardrop (Eds.), Schalm'S Veterinary Hematology, 6th edition, Wiley-Blackwell, Ames, Iowa, 2010, pp. 843–851.
[138] A.M. Perri, T.L. O'Sullivan, J.C.S. Harding, R.D. Wood, R.M. Friendship, Hematology and biochemistry reference intervals for Ontario commercial nursing pigs close to the time of weaning, Can. Vet. J. 58 (4) (2017) 371–376.
[139] D. Ventrella, F. Dondi, F. Barone, F. Serafini, A. Elmi, M. Giunti, N. Romagnoli, M. Forni, M.L. Bacci, The biomedical piglet: establishing reference intervals for haematology and clinical chemistry parameters of two age groups with and without iron supplementation, BMC Vet. Res. 13 (1) (2017) 23.
[140] C.A. Cooper, L.E. Moraes, J.D. Murray, S.D. Owens, Hematologic and biochemical reference intervals for specific pathogen free 6-week-old Hampshire-Yorkshire crossbred pigs, J. Anim. Sci. Biotechnol. 5 (1) (2014) 5.

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