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Selenium Requirements and Metabolism in Poultry

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Abstract

As counteract against deficiency in livestock, dietary treatments are supplemented with selenium (Se), usually as the inorganic form sodium selenite (SS). Since Se is considered as toxic as well as an essential element, SS is added to prevent an increase of Se in edible chicken parts. However, in many countries, populations suffer from suboptimal Se intake and even Se deficiency, by increasing the use of organic Se sources such as Se-enriched yeast or wheat in animal feed, there will be a subsequent increase in meat and egg products for human consumption. One could argue that the chickens do not need the extra pool of Se in muscles, as the inorganic form will be sufficient to meet the chickens Se requirements. Since the feed is fortified with selenite, the chickens will always have adequate access to the essential trace element. However, global gene set functional enrichment analysis revealed statistically significant enrichment of a number of biological processes that were dependent on the Se feed sources, such as cell growth, organ development and protein metabolism in favour of organic Se.

Keywords: selenium, requirements, metabolism, feed supplementation, selenoamino acids

1. Introduction

1.1. The element selenium

Selenium (Se) was discovered in 1817 by the Swedish chemist Jöns Jakob Berzelius [1, 2]. The element was named after the moon in Greek; Selene. Selenium is the chemical element number 34, a metalloid with six stable isotopes 76, 77, 78, 79, 80 and 82. The element is in the 16 group in the periodic table together with sulphur (S), oxygen (O) and tellurium (Te). These elements have

many common chemical properties. Selenium and S have similar electronegativities, atomic radius and the same oxidation states (-2, 0, +4, +6). Selenium is an S analogue, and substitute S in a series of compounds, such as sulphate (selenate), sulphite (selenite), sulphenic acid (selenic acid). Selenium and S react easily with each other forming selenylsulphide bounds [1]. Despite these similar properties, there are some major differences between S and Se, and substitution of one another results in different chemical properties. One of these differences is illustrated by the acid dissociation constant (pKa) value; H_2Se has a pKa of 3.73 and is a much stronger acid than H_2S with a pKa of 6.96 [1]. Another difference between the two elements is the reduction potential, under standard conditions Se is more readily reduced than S (e.g. SeO_4^{2-} has an $E = 1.15$ volt and SO_4^{2-} has $E = 0.158$ V).

1.2. Seleno amino acids

Cysteine (Cys) and methionine (Met) are S amino acids and both have Se analogs, SeCys and SeMet, respectively. As for the acids, the amino acids exhibit different chemical properties depending on the properties of Se and S. While the chemical properties of SeMet and Met are quite similar [3] the pKa (amino group) of SeCys is much lower than for Cys (5.2 vs. 8.3) [4]. At physiological pH the selenol group of SeCys will be in its anionic selenolate form, while the thiols of Cys residues are protonated. These properties make SeCys a more reactive amino acid compared to Cys [1], and give unique Se derived properties to selenoproteins.

1.3. Selenium in the terrestrial environment

High Se concentrations are found in sedimentary rocks and shales formed during the cretaceous period, while lower concentrations are characteristic for igneous (volcanic) rock, sandstone, granite and lime stone [5, 6]. The ubiquitous but uneven Se distribution in bedrock has resulted in Se concentrations in soil ranging from almost zero to $1250 \text{ mg Se kg}^{-1}$ in some seleniferous soils in Ireland [7, 8]. High soil Se concentrations can also be a consequence of prolonged deposition from precipitation containing seawater. In Norway the observed Se concentration in humus showed that the Se concentrations in soil decreases with distance from the sea [9]. Through observations in animal production, it is apparent that vast land areas worldwide do not supply sufficient Se for optimal livestock nutrition, and areas with low Se concentrations in soil are much more common than areas with very high Se concentrations in soil [8].

1.3.1. Selenium transfer from soil to plant

The distribution and availability of Se to plants depend on different soil conditions such as pH, organic matter [10], redox conditions, competing ionic species (e.g. sulphate), microbial activity, soil texture, compaction, mineralogy, temperature and moisture [11]. The bioavailability of Se from soil is more important than the total soil Se concentration. Inorganic Se species present in soil are selenide (Se_2^-), elemental Se (Se_0), selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) [12]. In aerobic soils, with pH around seven, selenate predominates, whereas selenite predominates at lower pH and redox potential. Under strongly reduced soil conditions selenide dominates [13]. Selenate is more mobile and thus available to plants compared to selenite.

1.3.2. Selenium in plants

The uptake of Se in higher plants depends on plant species and their physiological phase of development. Selenate is more accessible to plants than selenite and organic Se such as SeMet. The uptake of selenate and SeMet follow the active transporters of analog S species and is metabolized through the sulphate assimilation pathway. Selenite, however, is not mediated by membrane sulphite transporters [2]. After selenite has been taken up in the plant it is rapidly transformed to SeMet or SeCys and accumulated in proteins in the roots, while selenate is highly mobile in the xylem and translocated to plant parts above ground [12, 14]. The Se concentrations in plants are related to the general protein content of the plants and their different ability to accumulate Se. Most plants that are used in forage as well as grasses are non-accumulators [2] and, hence, have low Se concentrations. Surveys on Se concentrations in crop have revealed that areas producing crops with Se concentrations too low ($<0.1 \text{ mg Se kg}^{-1}$) to meet animal requirements are more common than areas producing toxic levels ($>2 \text{ mg Se kg}^{-1}$) of Se in crops, hence supplementation of diets is necessary to meet the animals Se requirements. There has been an increased interest of investigating the beneficial use of medical plants and phytochemical compounds in poultry diets [15], some of these plants are Se hyperaccumulators [16] and future research will prove if these plants could replace the traditional supplementation used today.

1.3.3. Selenium requirements in poultry and domestic animals

The toxicity of Se in animal diets was recognized around 1932 [17]. However, a decade later it was obvious that Se deficiency lead to several negative effect. Impaired growth and development, poor feathering, reduced egg production and hatchability, pancreatic degeneration, nutritional muscular dystrophy, and necrotic lesions in liver, muscle and heart are among the effects reported in Se deficient animals, all of which resulted in large economic losses for the industry [18–20]. In Finland, inadequate Se intake caused nutritional disorders in pigs and reduced the profitability of the production. As a result, all commercial animal feeds have been supplemented with selenite since 1969 [8], followed by Denmark in 1975, Norway in 1979 [21] and Sweden in 1980.

In addition, low Se levels reduce the immune function and the animals are more prone to develop diseases such as white muscle disease, cardiac muscle metamorphosis, blood capillary disease, cancer, anaemia and liver bleeding [22, 23]. In low Se areas worldwide, livestock producers have adopted methods that ensure adequate Se status in animals. Since selenium was foreseen as a toxic element, the supplementation recommendations were designed to ensure that the Se requirements were met without increasing the Se concentrations in tissues significantly. In that way humans were protected from ingesting “toxic levels” of Se from food products [24]. Sodium selenite (SS) and sodium selenate which do not increase muscle Se levels substantially were therefore approved as supplements to the livestock and poultry industry [24]. Selenite is most often used [24]. The use of Se-enriched yeast (SY) as feed additive is increasing. The European Food Safety Authority has approved a maximum of 0.2 mg Se from different yeast strains in complete Feed [25, 26]. Se-enriched yeast approved as supplement consists of 60–85% SeMet, 2–4% SeCys and $<1\%$ selenite or selenate [27]. Based on the legisla-

tion from the European Committee the allowed maximum total feed Se concentration is 0.5 mg kg⁻¹ DM (dry weight) [26]. These regulations also distinguish between inorganic or organic Se, where the maximum of organic Se is set to 0.2 mg Se kg⁻¹. Numerous reports on the positive effects of organic Se sources in animal feed are available. In addition to reduced frequency of diseases related to Se deficiency, organic Se readily increases tissue Se concentrations [28, 29]. In 2014 DL-Selenomethionine was accepted as an organic Se source after it was showed to be safe for chickens for fattening up to 1.5 mg Se/kg feed, the upper limit of 0.2 mg organic Se/kg feed still stands [30].

2. Understanding the speciation and metabolism of selenium in poultry

2.1. Bioavailability and bioaccessibility of selenium in a sample preparation and instrument analysis perspective

2.1.1. Speciation analysis of selenium

Speciation analysis of Se is applied to identify and quantify Se species present in different matrices. This is of major importance as bioavailability, cross membrane transport and metabolism of Se is highly dependent on the Se species present [31]. While numerous studies on Se species in yeast and Se accumulating plants have been published (e.g. [32–34]), fewer studies have been carried out on Se species in common food. It has therefore been necessary to develop reliable analytical techniques to study the speciation of Se in environmental and biological samples, to understand the biochemical cycle of Se [35, 36].

Selenium is usually present in food and biological samples at low concentrations and can be present as a variety of Se species. To perform speciation analysis of Se in biological matrixes different methodologies are applied. Hyphenated techniques such as liquid chromatography coupled to inductive coupled plasma mass spectrometry (LC-ICP-MS) and liquid chromatography coupled electrospray ionization mass spectrometry (LC-ESI-MS) are two complementary detection methods [37]. The ICP-MS quantifies based on signals from the elements mass/charge ratio; the ionization is virtually species independent and accurate absolute quantification is possible. In ESI-MS the Se species are identified based on molecules mass/charge ratio, where ionization is species and matrix dependent and quantification requires isotopically labelled species. Identification based on retention time matching with well-defined standards by HPLC-ICP MS is tentative, while ESI-MS data alone does not provide sufficient evidence for structural confirmation [33, 38].

With increased knowledge and sensitive instruments, more information on Se species in food will emerge. As many detectors are very sensitive (low detection limits), the challenges lie within sample preparation and chromatographic separations. At present, no standardized approaches to Se speciation analysis exist and different strategies of sample preparation and separation prior to detection are applied.

2.1.2. Sample preparation and separation techniques

Selenium species in food and biological samples can be extracted using different agents. Water extraction has been applied to water-soluble Se amino acids (MeSeCys and γ -glutamyl-MeSeCys), driselase is used to release Se bound to cell walls, different proteases for hydrolysis of selenoproteins and seleno-containing proteins [34], and tryptic digestion of water-soluble Se species are applied to identify peptide sequences [39]. Another approach is the use of in vitro digestion steps mimicking the gastro intestinal tract to get information on the bioaccessible fraction of Se from different matrices [40, 41].

Following extraction, different techniques are used to separate Se species. The most used technique is high performance liquid chromatography (HPLC) with reversed phase (RP), ion-pairing (IP)-RP, or ion-exchange (IE) columns, sometimes with a pre-concentration step using a size exclusion column (SEC) to collect the low molecular mass fraction [33, 42, 43]. Optimization of the chromatographic separation conditions to obtain narrow and well defined peaks are strived for at all times for best results [37]. Isotope dilution has improved the quality control of analysis and can be used species-specific or species unspecific, and be applied pre or post column to the ICP-MS [44].

During the last years, measurement of Se has focused on a more proteomic approach revealing information on selenoproteins and seleno-containing proteins. Examples are proteins being separated on gel electrophoresis (GE) (sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS PAGE) for protein identification by matrix-assisted laser desorption/ionization (MALDI)–time of flight (TOF)-MS or ESI-MS or laser ablation ICP-MS to identify the proteins containing Se in a gel [45]. Another way to identify Se species in a SDS PAGE is the use of radioactive labelled ^{75}Se . Neutron activation analysis (NAA) followed by gamma measurements of Se could give information on trace levels of Se in different matrixes. The use of affinity columns separating selenoalbumin (SelA), selenoprotein P (SelP) and glutathione peroxidase (Gpx) has been successfully reported [45].

Isotopic labelling of Se species makes it possible to follow the fate of different species in the environment, either applied in fertilizers to plants and vegetables, in feed or injected to animals. Detailed knowledge on the formation of different Se species has been gained by this approach [46–50], but some questions remain unanswered such as the turnover of Se from plant crops in adequately fed animals, or the uptake and transformation of Se from colon [36]. Speciation analysis of Se is essential to understand the uptake, metabolism, distribution and transformation of Se in biological system.

2.2. Selenium species dependent metabolism of selenium

According to [51] all Se-forms (chemically pure forms and different forms in food) are generally well transported over the intestinal membrane (70–95%), but the uptake varies according to the Se source and status of the individual [51]. The same active transporters take up selenate as sulphate, while selenite is taken up by passive transport and does not share the pathway of sulphite. Immediately after selenite has entered the red blood cells, it is reduced to selenide by cellular glutathione [52] or through the actions of thioredoxin reductase [53]. Thereafter,

selenide is transported to an organ, usually the liver, to undergo selenosynthesis [52]. Selenate reaches the liver in its intact chemical form where it is reduced to selenide through the same pathways as selenite [54]. In the liver of animals inorganic Se and organic Se is transformed into SeCys.

Organic seleno amino acids also reach the liver in their intact form where the conversion to selenide may follow different pathways. SeCys is transformed to selenide through the γ -lyase reaction, while SeMet can follow three known reaction pathways;

1. Conversion to SeCys through the intermediate SeCystathionine.
2. Directly by γ -lyase reaction.
3. SeMet does not enter the selenide pool, but is inserted into proteins as Met by tRNA coding for Met [50, 52, 53]. When SeMet substitutes for Met in proteins the Se concentration increases in the form of Se containing proteins.

Selenide is assumed to be the main precursor of Se metabolism. Selenide has three main conversion routes dependent on the Se status of the organism. Firstly, selenide is transformed to Se-phosphate then to Se-cysteyl tRNA for insertion of SeCys in selenoproteins. Secondly, at lower levels of intake, excess selenide not used in selenoprotein synthesis is also converted into selenosugars for excretion via urine. Thirdly, at high levels of intake, methyltransferases add a methyl group to selenide, leading to a sequential conversion to methylselenol and further to dimethyl selenide (excreted via breath and feces) and trimethyl selenonium (excreted via urine) in addition to excretion of selenosugars [50, 53, 55].

Methylated Se species, MeSeCys, γ -glut-MeSeCys and selenobetaine follow different pathways compared to the other dietary Se species described. The methylated selenospecies are transformed directly into methylselenol and excretion, or are demethylated to selenide [52].

Bioavailability studies should also address the transformation of Se into biological active metabolites (bioactive Se) [51, 52]. Selenoproteins are considered bioactive as they are essential for animals and humans.

Selenoprotein P (SeLP) is the major selenoprotein synthesized in the liver and is released to the blood stream, as a transporter of Se in the body. The liver also releases cellular Gpx to the blood stream whereas the kidneys release extracellular Gpx. According to [53] the uptake of SeLP from plasma is by specific receptor-mediated processes of apolipoproteins in brain and testis and megalin in kidneys. The specific uptake mechanisms to other tissues remain unknown.

The use of Se supplements to poultry improves the Se nutritional status by increasing the glutathione peroxidase Gpx activity in plasma. Glutathione peroxidase is the commonly used biomarker for Se status in livestock. When birds are fed with selenium enriched yeast the Gpx3 activity in blood remains higher for a longer period of time after supplementation than if the birds are supplemented with sodium selenite [56]. The Se supplementation and Gpx activity in poultry blood follow a dose response relationship [56–58], that seem to level off at approximately 400 ng/g whole blood [59].

2.3. Se supplementation of life stock

As mentioned earlier Se-enriched yeast increase chicken muscle Se concentrations significantly compared with sodium selenite [7, 57]. Hence, increasing the use of organic Se sources such as Se-enriched yeast in animal feed will give a subsequent increase in meat, egg and milk products for human consumption.

The aim of the present work was to compare the ability of Se-enriched wheat (SW), Se-enriched yeast (SY) and sodium selenite (SS), as Se supplements in dietary treatments to increase the Se concentration in edible chicken parts for human consumption. To compare chickens fed elevated Se dietary treatments with industrial produced chickens in Norway. The work also included identification and quantification of Se amino acids (SeMet, SeCys) in the edible parts of the chickens and effects on gene expression.

3. Animal experiment

The experimental research on animals followed internationally recognized guidelines. All animals were cared for according to laws and regulations controlling Norwegian experiments with live animals according to the Norwegian Animal Research Authority.

Ninety male chicken (*Gallus gallus*) (Ross 308, Samvirkelaget kylling, Norway) were divided into three groups and fed with either inorganic Se as sodium selenite (SS) or organic Se as Se-enriched yeast (SY) or Se-enriched wheat (SW). The calculated Se concentration in the dietary treatments was 0.8 mg Se kg⁻¹. Other main dietary constituents were added in equal amounts. Selenite and Se-enriched yeast were used as control groups to the Se-enriched wheat group, as these two Se supplements have been intensively studied at different concentrations levels [58, 60] among others. As a reference five chickens, 28 days of age, and chicken feed were collected from the chicken industry (Nortura SA chicken slaughterhouse in Rakkestad (Østfold, Norway; Ross 308). The industry use SS as the dietary source of Se, the maximum allowed concentration in the diet is 0.5 mg Se kg⁻¹ [27].

The chickens were fed ad-libitum for 33 days at the Animal Production Experimental Centre (SHF) at the Norwegian University of Life Sciences (UMB, Ås, Norway) randomly placed in separate pens. The animals were weighted after 8, 11 and 28 days. After 33 days the chickens were slaughtered, muscles (leg and breast) and liver sampled cut out, vacuum packed and stored frozen (-20°C) prior to analysis. Blood samples were taken by beheading and subsequent collection from the neck in 50 ml tubes (average volume of 30 ml), and stored at -20°C. The tissues were freeze dried prior to total Se measurements and speciation analysis.

3.1 Measurements

3.1.1 Total Se measurements

Tissue of muscles (breast and leg) and liver from chickens fed SS, SY or SW were freeze dried and homogenized with a mixer mill (Retsch mixer mill MM 200) equipped with zirconium

jars. The samples were digested using a microwave assisted (UltraCLAVE, Milestone) nitric acid (5 ml distilled suprapure 14 M HNO₃) decomposition of 0.1 g of dried tissue, or 1 ml (weight) of whole blood at 240°C for 40 min and diluted to 50 ml with 2% ethanol. Total Se was measured using high resolution inductive coupled plasma mass spectrometry (HR-ICP-MS) (Thermo Finnigan Element2, Bremen, Germany) at the National Institute of Occupational Health (STAMI). The instrument was used in high resolution mode. Tellurium was added as internal standard (IS).

The total concentration in the supernatants were digested (1 ml, weight) with sub-distilled nitric acid using the UltraCLAVE and diluted to 50 ml with MQ water. The samples were mixed 1:1 with 4% ethanol using a mixing block connected to the peristaltic pump of the ICP-MS (Perkin Elmer, Elan 6000) and total Se was measured on mass 82. Wheat flour (1567A) and Bovine liver (1577B) from NIST were used as certified reference material. Tellurium was added as internal standard (IS).

3.1.2 Speciation analysis

Proteolytic extractions were carried out according to the method from [61] on freeze dried and homogenized samples from three different chickens of each dietary treatment. One ml of 0.1 M Tris-HCl (pH 7.5) was added to 0.1 g meat sample and the suspension mixed with an ultrasonic probe for 1 min. Reduction and carboxymethylation (CAM) of the samples was done by adding 200 µl 0.2 M DTT (in 0.1 M Tris-HCl pH 7.5) and 275 µl 0.5 M IAM. The samples were incubated with careful mixing in the dark at room temperature (20°C). After 2 h 2.5 ml 0.2 M DTT was added and the samples shaken for 1 h in order to destroy excess of IAM. Then, 7 ml of 0.1 M Tris-HCl buffer was added together with protease XIV and lipase. The samples were digested with 30 mg protease and 20 mg lipase over night (×3) at 37 ± 0.5°C in an incubator cupboard on a Roto-Shake (Genie) in the dark. All digested samples were centrifuged at 10,000 × g for 10 min at 4°C and supernatants pooled and freeze dried. The freeze dried supernatants were dissolved in 5 ml of MQ water. One ml was used for total Se measurements and 4 ml for identification and quantification of Se amino acids. Enzymatic extracts were centrifuged at 12,110 g for 5 min (mini Spin, Eppendorf) and filtered through a syringe filter (0.45 µm) before high performance liquid chromatography (HPLC, Perkin Elmer 200 series pump)-ICP-MS (Elan 6000) analysis. The chromatographic separation was done on a reversed phase (RP) Altima C8/Alltech (150 × 4.6 mm, 5 µm) column using an injection volume of 100 µl. Isocratic elution of 2% MeOH and 0.1% HFBA (as an ion pairing agent) with a flow rate of 0.8 or 0.9 ml min⁻¹. Separation of CAM SeCys and CAM SeMet were preformed within 10 min.

The SeCys standard was synthesized according to the procedure described by [61]. Wheat flour (BC210a) from European Reference Material (ERM) from LGC-standards is verified for SeMet and was added to the speciation analysis for quality assurance.

3.1.3 Amino acid measurements

Total (peptide bound and free) amino acids were measured in two breast muscle samples from each treatment (SS, SY and SeW). The measurements were performed on a Biochrom 30 Amino

Acid Analyzer (Biochrom Ltd., Cambridge, UK) at Aquaculture Protein Centre (APC) at NMBU, following the procedure described by the Commission Directive 98/64/EC (1998). The samples were oxidized and hydrolyzed prior to HPLC-ultra violet (UV) measurements. The method does not distinguish between salts of amino acids and cannot differentiate between D and L forms of amino acids.

3.1.4 Gene expression analysis

Gene expression analyses offer a sensitive and rapid detection of transcriptional changes occurring at the cellular level after different treatments and exposures. Global (un-biased) transcriptional analysis using a oligo-array was applied to see whether SS and SW dietary treatments had different effect on the gene expression profiles of chicken muscle in regard to chicken health. The analysis was performed at Norwegian Institute of Water Research (NIVA).

Isolation of mRNA from fresh frozen muscle samples were performed by TRIzol[®] extraction, quality controlled by measuring salt and phenol interferences and approximately mRNA concentration by nanodrop (Nanodrop[®] ND-100 UV-Visible spectrophotometer, NanoDrop Technologies). The samples were diluted to 100 ng μL^{-1} so that RNA integrity could be determined by gel electrophoreses (Bioanalyzer Instrument, Agilent Technologies) samples passing the quality cut-off criteria had RNA Integrity Number (RIN) >9 , $260/230 > 1.99$ and $260/280 > 2.31$.

Microarray analysis was performed following Agilent's protocol "One Color Microarray-Based Gene Expression Analysis" (Quick Amp Labelling, Version 5.7 March 2008) using a 44k Agilent Chicken (V2) array.

3.1.5 Statistics

Statistical analysis was conducted using one way ANOVA with 95% confidence interval and Tukeys simultaneous test to establish statistical difference ($p < 0.05$) among individual treatment means using GraphPad prism version 5.

3.2 Results and discussion

3.2.1 Feed Se concentrations

The Se concentration in the reference diet from Nortura SA ($n = 6$) was 0.47 ± 0.052 mg Se kg^{-1} . The Se concentrations in the elevated dietary treatments ($n = 6$) were 0.90 ± 0.030 mg Se kg^{-1} for SS, 0.91 ± 0.040 mg Se kg^{-1} for SY, and 1.0 ± 0.017 mg Se kg^{-1} for SW. The Se concentrations were 0.1–0.2 mg Se kg^{-1} higher than the estimated concentration of 0.8 mg Se kg^{-1} , likely attributed to other constituents in the diet such as fishmeal and soy meal.

3.2.2 Bird physiological performance

The mortality was within the expected range (10% in the group fed with SS, 17% in the group fed with SY, and 3% in the group fed with SW) of experiments performed at the Animal

Production Experimental Centre. There were no effects of dietary treatment on intake or live weight gain.

3.2.3 Bioavailability of Se: total Se concentrations

The limit of detection was $0.4 \mu\text{g L}^{-1}$ based on three times the standard deviation of eight blank samples and the mean standard deviations of Se-77, Se-78 and Se-82. The limit of quantification was based on 10 times the standard deviation of the blank samples and was $1.3 \mu\text{g L}^{-1}$.

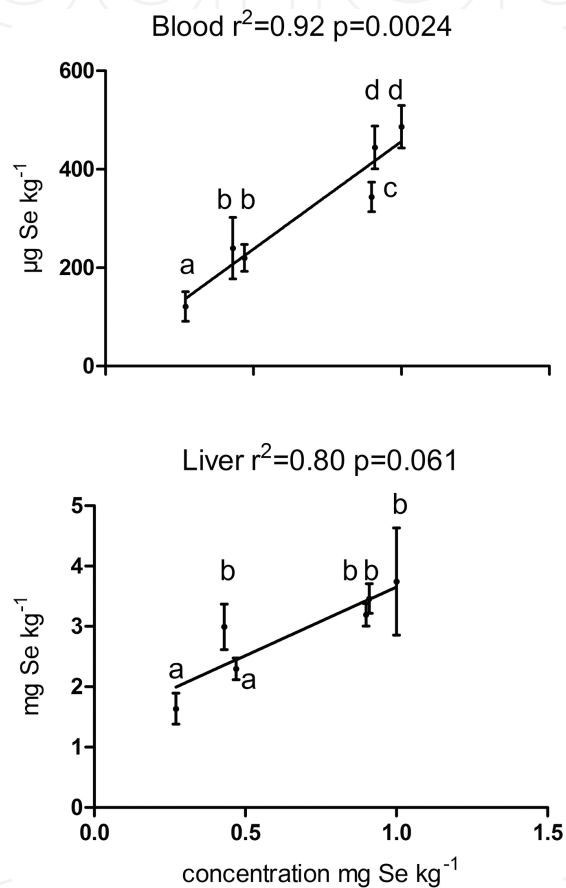


Figure 1. Correlation between feed Se concentration and liver and blood Se concentrations.

Whole blood (wet weight) Se-concentrations were significantly higher ($p < 0.05$) for all the chickens receiving Se elevated dietary treatments (SS, SY and SW) compared to the reference group (reference SS) (**Figure 1**). The Se concentration in whole blood from the chicken receiving SY and SW were $468 \pm 43 \mu\text{g Se kg}^{-1}$ and $521 \pm 43 \mu\text{g Se kg}^{-1}$, respectively and significantly higher ($p < 0.05$) than the concentration of $362 \pm 32 \mu\text{g Se kg}^{-1}$ in whole blood of the SS group. There were no differences in blood Se-concentrations between the two groups fed with organic Se. The dose dependent increase of Se in blood (**Figure 1**) with increasing Se in the diet is in line with previous published results in chickens and young turkeys [7, 57, 58]. The results are, however, not in line with results from [58, 59] where no difference in whole blood Se concentrations were observed between SS and SY at the same dietary Se level (in lamb and chickens,

respectively). The Se concentration in whole blood ($220 \pm 27 \mu\text{g Se kg}^{-1}$) of the reference group was in accordance with the concentration in whole blood ($240 \mu\text{g L}^{-1}$) of the SS group of [58] with a dietary treatment of $0.42 \text{ mg Se kg}^{-1}$.

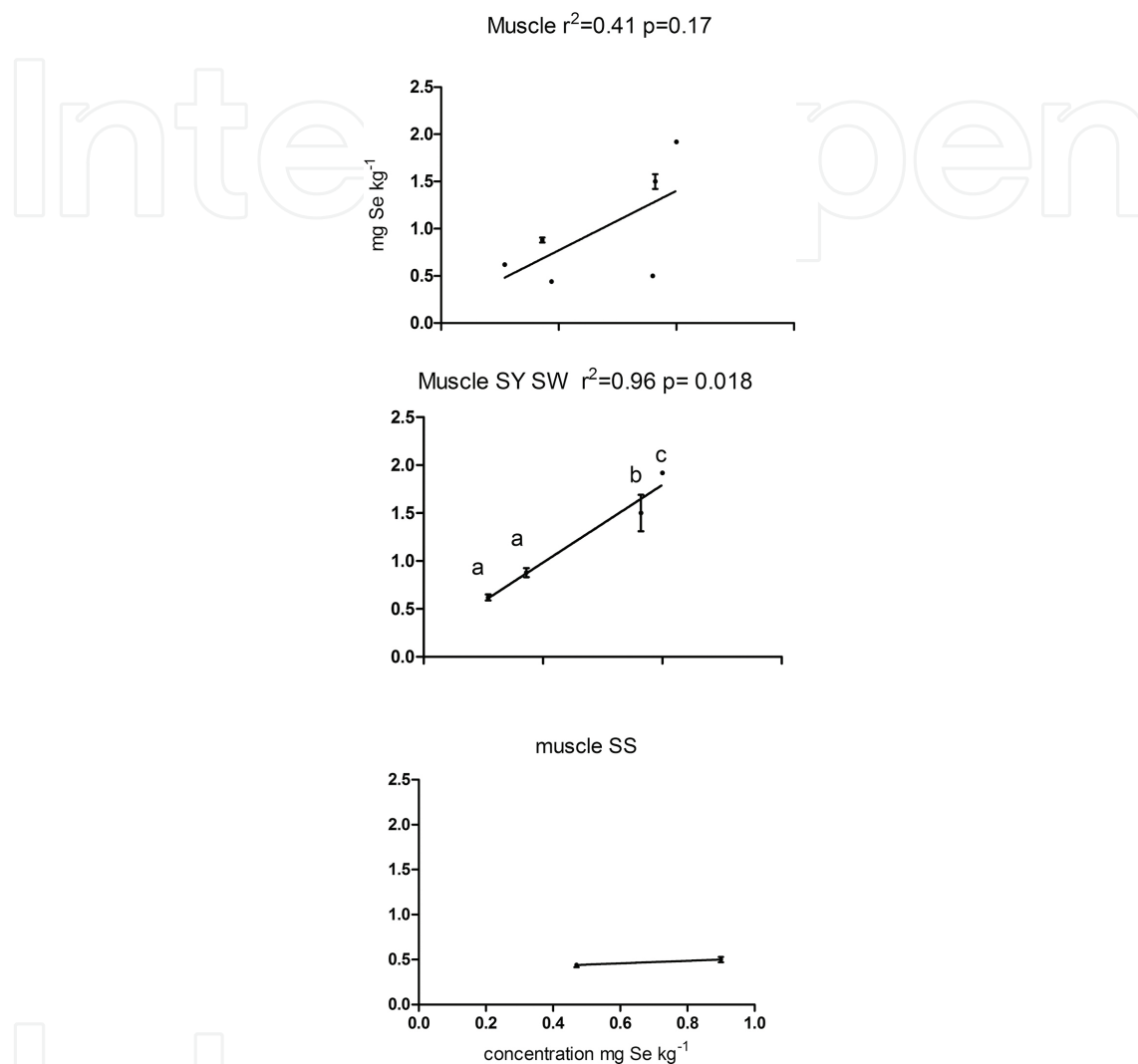


Figure 2. Correlation between Se concentration in feed and Se concentration in muscle with regard to Se source added to the feed (SY, Se-enriched yeast; SW, Se-enriched wheat and SS, sodium selenite).

The total Se concentration differed among tissue types; the liver had the highest Se concentrations, followed by the breast muscle and leg muscle (**Figure 2**). The high liver Se concentration is due to the tendency of glandular visceral tissue to have higher Se concentration than skeletal tissue [59], and the fact that the liver handles most of the absorption of nutrients and regulates their release into blood for further distribution or excretion. The Se concentrations in the livers of the reference group were $2.3 \pm 0.18 \text{ mg Se kg}^{-1}$. By increasing the Se concentrations in the diets to $0.9\text{--}0.10 \text{ mg Se kg}^{-1}$ the Se concentrations in livers increased significantly ($p < 0.05$) compared to the reference group (SS: $3.2 \pm 0.19 \text{ mg Se kg}^{-1}$, SY: $3.5 \pm 0.25 \text{ mg Se kg}^{-1}$, SW: $3.8 \pm 0.89 \text{ mg Se kg}^{-1}$) (**Figure 1**). The equal Se concentrations measured in liver of chickens

receiving dietary treatments with different Se forms at the same Se concentrations level was in accordance with results obtained by [49].

An increase from 0.47 ± 0.052 – 0.9 ± 0.030 mg Se kg⁻¹ of Se as SS in the diets did not result in a significant increase in the breast or leg muscle Se concentrations, 0.44 ± 0.24 and 0.50 ± 0.31 mg Se kg⁻¹, respectively (**Figure 2**). Se-concentration in chickens muscle of chickens fed with organic Se was significantly higher ($p < 0.05$) than in muscles from chickens fed with selenite. The Se concentration in breast muscles were significantly higher ($p < 0.05$) in the group receiving SW compared to SY (1.9 ± 0.020 and 1.5 ± 0.019 mg Se kg⁻¹). No difference between SW and SY diets on the total Se concentrations measured in leg muscle. Most publications on Se in dietary treatments use 0.3 and 0.5 mg Se kg⁻¹ (e.g. [57]). The muscle Se concentrations in the chickens receiving SS in the diet was in line with the concentrations reported by [62] after dietary supplement with 0.6 mg Se kg⁻¹ as SS and 0.6 mg Se kg⁻¹ as SY after 42 days, and in line with the total Se concentrations reported by [63] in breast muscle of Ross 308 chickens.

These data show that Se from SW result in similar Se concentrations in muscle as do SY, and based on these criteria SW could be an Se source for biofortification of Se in chickens, as suggested by [64]. The increased concentration of Se in muscle from organic Se in dietary treatments has been shown by several authors, and is attributed to the unspecific uptake of SeMet in the methionine pathway resulting in an unspecific storage of Se in muscle proteins [7, 64–66].

A wet weight portion of 170 g of chicken meat from the industrially produced chickens or selenite supplemented groups would give a contribution of 18 ± 1.3 µg Se, which is close to the values given by the Norwegian Food Table of 13 µg (170 g portion). The contribution from 170 g of chicken meat from chickens supplemented with SW or SY would be 67 ± 7 µg Se, and cover the new Se recommendations from the Nordic Nutrition Recommendations [67] of 50 µg Se/day for women and 60 µg Se/day for men.

The bioactive Se is defined as the fraction of Se from feed or food that is converted into biological active selenoproteins [52]. SeCys is incorporated into all selenoproteins and quantification of SeCys will therefore give a measure of the bioactive amounts of Se in different tissues. Hence, the reservoir of SeMet is not related to a greater bioactivity because of the unspecific incorporation into general body proteins were Se is not recognized by the organism for selenoprotein synthesis [52].

The extraction recovery after enzymatic digestion for the determination of Se-species in muscle was between 94 and 109%, whereas the extraction from liver was lower and varied from 77 to 103%, in feed the extraction was between 83 and 104%. The lower extraction efficiencies in some of the liver and feed samples may be related to a high fat content. The problems with the SeMet derivatization is likely the explanation for the low column recovery (~80%) of total Se compared to the extraction efficiency (>95%) in some of the samples, The different chemical structure of the Se binding to – H in the SeCys and – CH₃ could give different reaction with IAM. The derivatization of SeCys was fast and complete based on the occurrence of one narrow peak in the chromatogram by RP-ICP-MS and confirmation on ESI-MS of the CAM SeCys standard at mass to charge ratio (m/z) 227 showing the isotopic pattern of Se. ESI spectra of

CAM SeMet standard did not give the expected peak at 255 m/z, but rather a peak at 194, which may be explained by the loss of $-\text{CO}_2$ (44 m/z) and NH_3 (17 m/z) from CAM SeMet [43]. The RP-chromatograms of the muscle samples showed one distinct peak of SeMet identified by standard addition at 4 min, a smaller peak identified as protein bound SeCys after 2.7 min and four smaller unidentified Se species. In the liver samples CAM SeMet eluted in two peaks (retention time of ca. 5.4 and 5.9 min) and protein bound CAM SeCys at 4 min, as for the muscle samples four smaller unknown peaks were observed in the chromatograms.

3.2.4 Se amino acid concentration in liver and muscle

SeMet was the predominant Se amino acid in livers of the chickens receiving dietary treatments with SW or SY, whereas SeCys was the dominating Se amino acid in livers of chickens receiving SS as dietary supplement. These results are not in line with the findings of [57] where SeCys was predominant in liver tissue irrespective of treatment (with feed Se concentrations of 0.3 and 0.5 mg Se kg⁻¹). On the other hand in experiments with lamb [59] and beef cattle [59] receiving SY doses tend to result in SeMet comprising the greater proportion of total Se in the liver tissue which is in accordance with the result in the present work using elevated Se concentrations in the diet (**Figure 3**).

The different Se amino acid portioning may reflect a saturation of SeCys in the liver at higher SW or SY doses and subsequent change from incorporation into selenoproteins to the non-specific incorporation of SeMet into general liver tissue proteins [57].

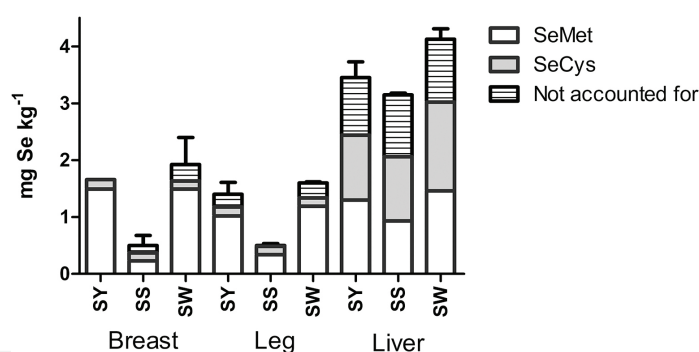


Figure 3. Concentration of Se amino acids in muscle and liver with regard to Se source (SY, Se-enriched yeast; SW, Se-enriched wheat and SS, sodium selenite).

The predominance of SeMet in breast tissue of chickens receiving SW or SY in the dietary treatment is in line with the observations of [42, 57].

The concentration of SeCys in breast and leg muscle tissues were irrespective of dietary treatment ($0.15 \pm 0.02 \mu\text{g Se g}^{-1}$; $n = 6$) and in accordance with concentrations reported by [42], where 3 (0.11 ± 0.0 , 0.18 ± 0.01 and $0.13 \pm 0.08 \mu\text{g Se g}^{-1}$) out of 4 ($1.11 \pm 0.35 \mu\text{g Se g}^{-1}$) samples from chicken breast tissue had similar Se concentrations. It seems that the source of Se did not affect the concentration of SeCys in the muscle when there is an excess or adequate concentration of Se in the diet. The low level of SeMet found in tissues of chickens fed with selenite is likely due to other constituents in the diet, e.g. fishmeal and soybean [68]. The Se speciation

analysis showed that the SeMet concentration was a factor 5 higher in chickens fed organic Se than in chickens fed with selenite. The present results show that Se from SS was less retained in muscle tissues compared to Se from SY and SW, and the retention observed is attributed to the incorporation as SeCys in proteins as stated by [65]. An increased organic Se source consisting primarily of SeMet would increase the SeMet concentration in the tissues and thereby the retention of Se in muscle tissue.

Even though the concentrations of Se in the liver were the highest, the effect on daily human intake would be of minor significance as the consumption of liver is low compared to the consumption of breast and leg muscle. Therefore focus should be on the increase of Se concentration in chicken breast and leg muscle by feeding organic Se to the chickens (SY and SW). The bioaccessibility of Se from the chicken meat to humans has previously been investigated by an in vitro model [41], showing relatively high accessibility of Se from chicken meat to humans (70–90% depending on the method used). Furthermore, human studies with isotopic labelled Se have demonstrated bioavailability over 90% from different food sources [69]. The Se biofortification of edible chicken meat products will increase the human Se intake and should be considered as a relevant strategy to increase the Se concentration in human plasma.

3.2.5. Amino acid composition

The use of organic and inorganic Se in the feed did not affect the overall amino acid distribution in the breast muscle tissues. The composition of amino acids in chicken meat is highly dependent on the composition of the diet. In this experiment the diets were added methionine (Met) (3 g kg^{-1}), lysine (Lys) (4 g kg^{-1}) and threonine (Thr) (1 g kg^{-1}), other important protein sources in the feed was soybean and fish meal. According to [63] a concentration of 3 g Met kg^{-1} will affect the bioavailability of SeMet and transfer to muscle, as the two amino acids are taken up by the same sodium transport system. This system is specific for neutral amino acids, resulting in inhibition of SeMet by Met as there is only trace concentration of SeMet compared to Met in the diet.

The amino acid distributions were compared to the Danish Food Composition Databank (DFCD) (Ed. 07.01) and What's In The Food You Eat Search Tool from United State Department of Agriculture, the amino acid composition of the breast muscles were comparable to what is reported in the DFCD but not from USA. This is probably because the Danish and Norwegian diets are wheat based whereas the diets in USA are maize based. The knowledge of the interaction between Se and Met is limited [63] and since all diets had the same Met concentrations it is impossible to extract any information on the influence on SeMet in this experiment.

3.2.6. Gene expression of muscle samples

Muscular gene expressions in *Gallus gallus* as a result of inorganic versus organic Se supplement (SS and SW) were determined with the use of a 44k Chicken oligonucleotide microarray. The results indicated similar expression of genes coding for selenoproteins, which is in line with the finding of equal SeCys concentrations in the muscles. However, global gene set

functional enrichment analysis (Fatiscan) revealed statistically significant enrichment ($p < 0.05$) of a number of biological processes that were dependent on the Se feed sources, such as cell growth, organ development, protein metabolism (**Table 1**). Although Bayers statistics were not able to detect changes in differential expression of single genes, fatiscan analysis based on ranking of statistical values from these gene-centric analysis were able to identify subtle changes affecting a broad set of biological responses. Future effort to elucidate the biological meaning of the findings may potentially provide a mechanistic understanding to characterize molecular signatures associated with organic and inorganic Se supplements.

Functional term	Score*
Anatomical structure development	5.67
Organ development	4.34
Anatomical structure morphogenesis	2.79
Protein catabolic process	2.31
Nervous system development	2.18
Nitrogen compound metabolic process	2.18
Skeletal system development	1.96
Cellular nitrogen compound metabolic process	1.96
Regulation of cell size	1.96
Cell growth	1.6
Brain development	1.6

*A term annotation weight can be computed as the number of sequences annotated to that term or as an annotation confluence score. This confluence score (Node Score) takes into account the number of sequences converging at one gene ontology term and penalizes by the distance to the term where each sequence actually was annotated.

Table 1. Differences in regulations of genes in biological processes in chicken muscle of chickens fed SW ($n = 4$) compared to muscle of chickens fed SW ($n = 4$).

By comparing the fold change of genes involved in Se processes, it could be deduced that the use of Se-enriched wheat positively regulated developmental and metabolic processes in the muscle compared to selenite. The difference may be due to differences in uptake and bioavailability interacting (stimulatory/suppression) with biological processes. Brennan et al. [70] reported similar findings in oviducts of female chickens indicating differences in growth and metabolic patterns being higher when supplemented with SY compared to SS at lower Se concentrations ($0.3 \text{ mg Se kg}^{-1}$).

4. Conclusion

Organic Se is three times more efficient than inorganic Se in increasing the chicken breast and leg muscle Se concentration, due to the high accumulation of SeMet in muscle proteins. The Se-source neither affects the muscle SeCys concentration nor the amino acid composition in the muscles. The use of SW as dietary supplement in chicken feed is an alternative to the SY based supplements to increase Se and in particular SeMet concentrations in edible chicken

parts. The bioactive Se is defined as the fraction of Se from feed or food that is converted into biological active selenoproteins. SeCys is incorporated into all selenoproteins and quantification of SeCys will therefore give a measure of the bioactive amounts of Se in different tissues. A relatively high proportion of the Se from the diets were converted into bioactive SeCys in the liver ($35 \pm 2.2\%$), no significant difference between inorganic and organic Se sources. The concentration of SeCys in breast and leg muscle tissues were irrespective of dietary treatment. The results indicated similar expression of genes coding for selenoproteins, which is in line with the finding of equal SeCys concentrations in the muscles.

Due to longer retention time of SeMet in muscle, and possibly other organs, SeMet acts as a reservoir of Se in the body. The SeMet reservoirs could sustain the SeCys status of the chickens over time [57]. To our knowledge there is no known biological functions of SeMet, and the inorganic forms are readily transformed to SeCys through selenide in liver. One could argue that the chickens do not need the extra pool of Se in the form of SeMet in e.g. muscles, as the inorganic form will be sufficient to meet the chickens Se requirements. Since the feed is fortified with selenite the chickens will always have adequate access to the essential trace element. However, global gene set functional enrichment analysis revealed statistically significant enrichment of a number of biological processes that were dependent on the Se feed sources, such as cell growth, organ development and protein metabolism in favour of organic Se.

Selenium from wheat or chicken muscle could be important Se sources to ensure adequate Se intake in humans in Se sub deficient or deficient populations.

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