Comparing responses to different selenium sources and dosages in laying hens

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ABSTRACT Developing new sources of organic Se has potential benefit for animal production and human nutrition via animal-based foods enriched in Se. The objectives of this trial were to compare L-selenomethionine with another organic Se source, Se-enriched yeast (SelPlex 2300), and sodium selenite, an inorganic Se source, against a commercial control diet. The effect of source and the dosage of Se supplementation on Se in eggs and blood variables was investigated. Ten treatments were used with 18 laying hens per group. In addition to the control diet, the control diet was supplemented with L-selenomethionine, Se-enriched yeast, or sodium selenite at 0.1, 0.3, or 0.5 mg/kg of Se. The feeding trial lasted 8 wk. Birds in the different treatment groups all showed good performance. At d 0 and 56, Se and glutathione peroxidase (GPx) were analyzed in 10 blood samples per group. After supplementing the diets for 56 d, significantly higher Se levels in serum and egg contents were reached for the Se-supplemented groups compared with the control. Supplementing 0.3 and 0.5 mg/kg of L-selenomethionine or Se-enriched yeast instead of 0.1 mg/kg significantly increased the serum Se levels, whereas no significant increase was found for sodium selenite. No effect of Se source or dosage was observed on serum GPx levels. Selenium in eggs was significantly affected by dosage and source of Se. The Se supplementation level in the feed was reflected in the eggs, with the highest and lowest values for 0.5 and 0.1 mg/kg, respectively, and values in between for the 0.3 mg/kg supplementation level. A dose response was most pronounced for L-selenomethionine, followed by Se-enriched yeast, and was least when Se was added as sodium selenite. It can be concluded that Se from organic sources was more bioavailable than the inorganic Se source as evidenced by blood and egg Se levels. Within the organic Se sources, L-selenomethionine showed higher Se transfer to eggs than Se-enriched yeast.

Key words: laying hen, selenium, dosage, source

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INTRODUCTION

Selenium is an essential mineral for animal nutrition and plays an important role in immune function, health, and productivity. This essential trace mineral is also of fundamental importance to human health. As a constituent of selenoproteins, Se has structural and enzymic roles, being best known in the latter context as an antioxidant, as the Se-dependent glutathione peroxidase defends the body against oxidative stress and is a catalyst for the production of active thyroid hormone. In summary, Se-enriched feed for poultry can improve the production performance of the birds and can also improve their immunity and antioxidant status (Surai and Fisinin, 2014).

Traditionally, Se has been added to poultry diets via inorganic sources, such as sodium selenite (**SS**; Na_2SeO_3). Organic sources of Se, such as Se-enriched yeast, have been explored as an alternative to inorganic supplementation (Payne and Southern, 2005). Research has shown that organic Se is more bioavailable than Se in SS (Payne et al., 2005). Compared with inorganic Se, organic Se leads to higher rates of absorption, tissue accumulation, and antioxidant activities, and to lower toxicities (Kim and Mahan, 2001) and less environmental pollution (Kuricova et al., 2003). This explains the increasing interest in organic Se during recent years. Many studies indicate that selenomethionine accounts for the largest portion of Se in Se-enriched yeast (Paton et al., 2002; Schrauzer, 2006; Upton et al., 2008). Most of the different sources of Se-enriched yeast have an average of 63% Se as selenomethionine. However, data obtained from practice indicate a wide variation in selenomethionine-Se concentration among sources of

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Se-enriched yeast (Schrauzer, 2006; Zhan et al., 2011). Dietary selenomethionine can be incorporated directly and nonspecifically in proteins, such as albumin, muscle tissue, eggs, and milk, instead of methionine. Alternatively, it can be trans-selinated to selenocysteine. Selenocysteine and other organic Se compounds in selenized yeast are transferred to hydrogen selenide (Combs, 2001; Rayman, 2004; Burk et al., 2006). Also, inorganic Se (such as SS) is transferred to hydrogen selenide (H_2Se), which in turn may be converted to selenophophate to generate selenoproteins or to be excreted.

Organic Se, in the form of selenomethionine, can be used to produce selenoproteins as well as general proteins containing methionine. Selenomethionine thus forms a Se reserve, whereas Se from inorganic supplements is only present in selenoproteins. It is hypothesized that the development of a new source of organic Se, L-selenomethionine, has potential benefit for animal production and human nutrition via Se-enriched foods of animal origin.

Therefore, the objective of this experiment was to compare different sources (inorganic and organic) and inclusion rates of Se on blood variables and Se content in the eggs of laying hens.

MATERIALS AND METHODS

Birds and Housing

Medium-weight laying hens (Lohmann Brown), 55 wk of age at the start of the experiment, were used in this trial. The birds were housed in the poultry experimental facility of the Institute for Agricultural and Fisheries Research (**ILVO**). In each pen unit, 18 laying hens were housed in 2 enriched cages (9 hens/cage) and reared under conventional conditions for lighting, heating, and ventilation. Drinking water and feed (as finely ground meal) was provided ad libitum. Duration of the trial consisted of a 4-wk adaptation period and an 8-wk experimental period. The trial consisted of 10 treatments, with 18 laying hens per experimental group for a total of 180 birds.

Dietary Treatments

The laying hens received a nonsupplemented Se basal layer (control) diet during the adaptation period. The basic feed was a wheat-corn and soybean meal diet without Se supplementation. A Se-free premix was formulated to contain all constituents except Se. The wheat-corn laying hen diet was formulated to contain adequate nutrient concentrations as recommended by Aviagen (2009) except Se (Table 1).

After this adaptation period, laying hens were distributed to the different dietary treatments based on performance to obtain similar groups. The trial was designed as a randomized complete block design with 10 dietary treatments. The experiment consisted of the nonsupplemented Se basal diet (control) and the basal diet (control) supplemented with 1 of 3 Se sources at different inclusion levels. The test products: L-selenomethionine (Excential Selenium, L-selenomethionine on a carrier of limestone, Orffa Additives, Werkendam, the Netherlands), Se-enriched yeast (SelPlex 2300, Alltech USA, Lexington, KY), and SS were provided by Orffa Additives B.V., the Netherlands. The Se sources were included at a rate of 0.1, 0.3, and 0.5 mg/kg. The different treatments were **Tr1** = control (not Se supplemented), **Tr2** = L-SeMet-supplemented diet at 0.1 mg/kg, **Tr3** = L-SeMet-supplemented diet at 0.3 mg/kg, **Tr4**

Table 1. Composition of the basal diet (control diet)[1]

Item	Value, %
Feedstuff	
Wheat	20.0
Corn	40.2
Soybean oil meal (48% CP)	18.9
Heat-treated full fat soybeans	7.1
Calcium carbonate (pelleted)	4.5
Calcium carbonate (powder)	4.2
Rendered animal fat	1.9
Bicalcium phosphate	1.7
Sodium chloride	0.2
Sodium bicarbonate	0.3
DL-Methionine	0.1
Carophyll yellow	0.004
Carophyll red	0.002
Nonstarch polysaccharides enzyme	0.02
Vitamin and trace element premix[2]	1.0
Nutrient composition[3]	
ME_n of layers, MJ/kg	11.9
CP, %	16.7
Calcium, %	4.05
Phosphorus total, %	0.63
Sodium, %	0.15
Chloride, %	0.16
Na^+K-Cl, mEq	216
dLysine, %	0.72
dS amino acids, %	0.60
dThreonine, %	0.51
Linoleic acid (C18:2), %	1.85

[1]Analyzed dosages of Se (mg/kg) of the different dietary treatments are as follows: control diet = 0.25 mg/kg, L-Se methionine (L-SeMet) supplemented diet at 0.1 mg/kg = 0.32 mg/kg, L-SeMet-supplemented diet at 0.3 mg/kg = 0.50 mg/kg, L-seleniomethionine-supplemented diet at 0.5 mg/kg = 0.71 mg/kg, Se-enriched yeast-supplemented diet at 0.1 mg/kg = 0.29 mg/kg, Se-enriched yeast-supplemented diet at 0.3 mg/kg = 0.50 mg/kg, Se-enriched yeast-supplemented diet at 0.5 mg/kg = 0.77 mg/kg, sodium selenite-supplemented diet at 0.1 mg/kg = 0.42 mg/kg, sodium selenite-supplemented diet at 0.3 mg/kg = 0.54 mg/kg, sodium selenite-supplemented diet at 0.5 mg/kg = 0.89 mg/kg.

[2]Composed of calcium carbonate, wheat, magnesium oxide, wheat feed, and as nutritional additives: vitamin A E672 (1,349,997 IE/kg), vitamin D_3 E671 (299,999 IE/kg), choline/choline chloride (60,000 mg/kg), vitamin E 3a700 (all-*rac*-alpha-tocopheryl acetate; 5,488 mg/kg), nicotinic acid/nicotinic acid amide (3,000 mg/kg), vitamin B_3/calcium D-pantothenate (1,500 mg/kg), vitamin B_2/riboflavin (500 mg/kg), vitamin B_6/pyridoxine hydrochloride (Ea831; 400 mg/kg), vitamin K_3 (250 mg/kg), vitamin B_1/thiamine mononitrate (200 mg/kg), folic acid (100 mg/kg), biotin/D-(+)-biotin (20 mg/kg), vitamin B_{12}/cyanocobalamine (2 mg/kg), manganese (II) oxide–manganese E5 (9,590 mg/kg), zinc (II) oxide–zinc E6 (6,000 mg/kg), ferrous sulfate (monohydrate)–iron E1 (4,920 mg/kg), cupric sulfate (pentahydrate)–copper E4 (2,000 mg/kg), calcium iodate (anhydrous)–iodium E2 (120 mg/kg), technological additives: ethoxyquine E324 (3,342 mg/kg), butylhydroxytoluene E321 (40 mg/kg), propyl gallate E310 (12 mg/kg), citric acid E330 (69 mg/kg), sepiolite E562 (400 mg/kg).

[3]dLysine = digestible lysine; dS amino acids = digestible sulfur-containing amino acids; dThreonine = digestible threonine.

= L-SeMet at 0.5 mg/kg, **Tr5** = Se-enriched yeast-supplemented diet at 0.1 mg/kg, **Tr6** = Se-enriched yeast-supplemented diet at 0.3 mg/kg, **Tr7** = Se-enriched yeast-supplemented diet at 0.5 mg/kg, **Tr8** = SS-supplemented diet at 0.1 mg/kg, **Tr9** = SS-supplemented diet at 0.3 mg/kg, and **Tr10** = SS-supplemented diet at 0.5 mg/kg.

The diets were produced at the ILVO experimental feed milling facilities. The experiment was conducted in accordance with the principles and specific guidelines in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Measurements

Performance. Feed intake was recorded and feed conversion and daily feed intake per bird were calculated for each 4-wk period. The number of eggs per pen unit was counted daily, and eggs were weighed every 2 wk. The incidence of cracked, soft-shelled, and dirty eggs was also recorded daily.

Twice a day, birds and housing facilities were inspected for the general health status, constant feed and water supply, temperature and ventilation, dead birds, and unexpected events. Daily mortality and cullings were recorded for each pen. Corrections for mortality calculating zootechnical performances were done using the number of bird days (number of birds × days alive). At the beginning of the trial (d 0) at d 28 and at the end (d 56), the laying hens were weighed.

Se Concentrations in Feed and Egg Contents. Total Se concentrations of the feed samples were determined. Homogenized feed samples (0.250 g/replicate) were mineralized in 4 mL of HNO_3 (extra pure, 65%) and 4 mL of double-distilled water in closed vessels (TFM, 50 mL) in a microwave oven (CEM MARS XPress, Matthews, NC). The vessels were heated to 180°C in 15 min, and this temperature was maintained for 30 min. After cooling, the solution was diluted (dilution factor 400) and ^{77}Se concentrations were determined by inductively coupled plasma-mass spectrometry (Varian 820, Varian, Melbourne, Australia) with H_2 as a reaction gas. The certified reference material SELM-1 (Se enriched yeast) accompanied each set of feed samples. Measurement uncertainty (k = 2) of the method is 32%, and limit of quantification (**LOQ**) = 80 $\mu g \cdot kg^{-1}$.

At d 0 and 56, 10 egg contents per treatment were analyzed for Se concentration. Eggs were mineralized with concentrated HNO_3 in a heating block (DigiPrep; 0.5 g sample + 2 mL of HNO_3 extra pure, 65%). The temperature program was as follows: 10 min at room temperature, gradually over 30 min increased to 60°C, held at 60°C for 30 min, gradually increased over 30 min to 105°C, and held at 105°C for 2 h. After mineralization, the samples were diluted (dilution factor 200) and Se concentrations were determined as described above. The certified reference material TORT-2 (lobster hepatopancreas) accompanied each set of egg samples. Measurement uncertainty (k = 2) of the method is 18%, and LOQ = 40 $\mu g \cdot kg^{-1}$. All samples were analyzed fresh, and results are expressed on a fresh-weight basis.

Plasma Analysis. At d 0 and 56, ten blood samples were taken per treatment group (serum and unclotted blood). Samples were analyzed for Se and glutathione peroxidase (**GPx**). Selenium concentrations in blood were determined via atomic absorption spectroscopy. The atomic absorption spectroscopy atoms, which are in the ground state, are irradiated with monochromatic light that is absorbed. A lamp is used with a line spectrum, namely, the hollow cathode lamp. Serum is diluted, and is dried, ashed, and atomized in the device. Selenium concentration is measured at a wavelength of 196. The intensity of the light before and after passage through the absorbent medium is measured. Then, a quantitative relationship is made between the measured absorbance and the number of absorbing atoms, or the atomic concentration of the element in the atomized sample. This process is done according to the law of Lambert-Beer. This law describes the relationship between absorbance and concentration. The LOQ = 10 $\mu g/L$, SD = 2.6, and CV = 16.2%.

Levels of plasma GPx were measured spectrophotometrically in samples collected at d 0 and 56 using a commercial RANSEL kit (RANDOX Laboratories Ltd., London, UK) according to the manufacturer's instructions. Briefly, GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NAD phosphate (**NADPH**), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. The minimum detectable concentration is determined as 74 U/L, SD of 17.5, and CV of 7.30%.

Statistical Analysis

Data were analyzed using the STATISTICA software program (Statistica 64.0, StatSoft Inc., 2012, Tulsa, OK). The PROC FREQ and PROC MEANS procedures were used for descriptive analyses. The assumption of normality of the outcomes was assessed applying stem and leaf plots and normal probability plots. The distribution of the percentage data was skewed. Therefore, an arcsin transformation of the percentage data was applied to obtain a normally distributed data set.

All treatments without the control treatment were compared by ANOVA performed with the PROC MIXED procedure with 2 fixed factors (source and dosage) and their interactions using the GLM procedure for blood variables and Se egg contents. Laying hen and egg were taken as experimental unit for the plasma and egg content analysis, respectively. Significant differences between treatments were separated using least squares means procedures. All statements of significance were based on a probability of 0.05. Regression analysis was used to determine linear effects of Se addition on Se concentrations in serum and egg contents.

RESULTS

Experimental Feeds

Analysis of the experimental diet indicated that Se from basal ingredients provided 0.25 mg/kg of Se for the control diet. Analysis of the Se-free premix indicated that a mean value of 8,978 (mean value of 2 analyses: 9,934 and 8,022 μg of Se/kg of premix) μg of Se/kg of premix was present. These values indicated a higher than expected level of Se present in the Se-free premix. Consequently, the Se level in all the dietary treatments was thus higher than intended. Results of the analyzed Se concentrations are presented in the footnote of Table 1. When taking the Se level of the C diet into account, the analyzed Se levels fitted well with the calculated Se levels. Analysis of Se-enriched yeast showed 2,272 mg/kg of Se and 1,568 mg/kg of Se as selenomethionine, and the L-selemethionine product on carrier (premix) showed 1,285 mg/kg of total Se and 1,135 mg/kg of Se as selenomethionine. It should be noted that the analysis for selenomethionine is not yet validated, so no LOQ, SD, or CV can be given.

Performance of the Laying Hens

The main results of the zootechnical performance of the layers during the trial of 2 × 28 d are presented in Table 2. Because no repetitive studies were carried out, no statistical analysis of the effect of the experimental feed on these parameters per period could be performed. In general, it can be stated that laying percentage ranged from 71.5 to 91%, egg mass from 51.6 to 60.1, feed intake from 108 to 117.1 g/d per hen, and feed conversion from 1.9 to 2.3.

Due to the low laying percentage, the daily egg mass of laying hens fed Tr4 and Tr7 was numerically lower compared with their counterparts. Therefore, feed conversion of these groups reached the highest value of all treatments. In general, it can be stated that birds in the different treatment groups all showed good performance (Table 2). The effect on the incidence of cracked, soft-shelled, and dirty eggs (Table 3) are presented per dietary treatment. The incidence of cracked or soft-shelled eggs was comparable between treatments, but percentage of broken eggs were numerically highest for Tr7 (Table 3).

Mortality was low during the trial period. In the L-selenomethionine treatment groups, 4 of 54 birds died; in the selenized yeast treatment groups, 3 of 54; and in the SS treatment groups, 1 of 54 birds died. No autopsies were performed. Due to the low number of birds per treatment, no statistical conclusions can be drawn about mortality (data not shown).

Se Concentration in Serum and Egg Contents

At the start of the experiment (d 0), no significant differences ($P > 0.05$) between dietary treatments were present because Se levels in serum as well as in the egg contents were comparable between dietary treatments (data not shown). However, at d 56 significant effects of dietary treatment on Se concentrations in egg contents as well as serum were found. Mean Se levels were significantly lower for the control group compared with the other dietary treatment groups (data not shown). Supplementing 0.5 mg/kg of L-selenomethionine resulted in a 2.4- and 3-fold increase compared with the C group for Se concentrations in the serum and egg contents, respectively. For the Se-enriched yeast supplementation, levels in serum and egg contents were increased by a factor of 2.3, whereas for SS this increase was 1.9 and 1.5 for serum and egg contents, respectively.

Furthermore, a dose response effect on the mean Se level in serum and egg contents was observed for the 3 Se sources because a higher dosage of Se in the feed resulted in a higher Se level in the serum ($P < 0.001$) and the egg contents ($P < 0.001$; Table 4) of the laying hens. In the Se serum levels, however, this effect was more pronounced for L-selenomethionine and Se-enriched yeast than for SS (source × level interaction,

Table 2. The effect of dietary treatment on laying rate (%), egg weight (g), daily egg mass (g), daily feed intake (g/bird), and feed conversion rate

Treatment	Laying rate, %	Egg weight, g	Daily egg mass, g	Daily feed intake, g/bird	Feed conversion
1 = Control	88.5	64.8	57.4	113.4	1.98
2 = L-SeMet 0.1	91.0	66.1	60.1	114.2	1.90
3 = L-SeMet 0.3	84.6	67.0	56.7	119.8	2.12
4 = L-SeMet 0.5	77.5	65.0	50.4	111.9	2.22
5 = Se-yeast 0.1	89.1	65.7	58.5	117.1	2.00
6 = Se-yeast 0.3	79.9	65.7	52.5	110.5	2.10
7 = Se-yeast 0.5	71.5	67.4	48.2	111.1	2.30
8 = Na-Se 0.1	79.0	65.3	51.6	108.0	2.09
9 = Na-Se 0.3	87.3	66.4	58.0	112.3	1.94
10 = Na-Se 0.5	87.8	67.1	59.0	114.6	1.94

[a–c]Means with the same letter are not significantly different from each other at $P \leq 0.05$.

[1]Control (no Se added), L-SeMet 0.1 = L-selenomethionine-supplemented diet at 0.1 mg/kg, L-SeMet 0.3 = L-selenomethionine-supplemented diet at 0.3 mg/kg, L-SeMet 0.5 = L-selenomethionine-supplemented diet at 0.5 mg/kg, Se-yeast 0.1 = Se-enriched yeast-supplemented diet at 0.1 mg/kg, Se-yeast 0.3 = Se-enriched yeast-supplemented diet at 0.3 mg/kg, Se-yeast 0.5 = Se-enriched yeast-supplemented diet at 0.5 mg/kg, Na-Se 0.1 = sodium selenite-supplemented diet at 0.1 mg/kg, Na-Se 0.3 = sodium selenite-supplemented diet at 0.3 mg/kg, Na-Se 0.5 = sodium selenite-supplemented diet at 0.5 mg/kg.

Table 3. Percentage of cracked, soft-shelled, and dirty eggs of 18 laying hens per treatment during a period of 8 wk[1]

Treatment	Cracked eggs, %	Soft-shelled eggs, %	Dirty eggs, %
1 = Control	3.0	0.0	0.0
2 = L-SeMet 0.1	0.9	0.0	0.2
3 = L-SeMet 0.3	2.9	0.1	0.4
4 = L-SeMet 0.5	5.5	0.3	0.5
5 = Se-yeast 0.1	3.3	0.2	0.1
6 = Se-yeast 0.3	4.3	0.5	0.3
7 = Se-yeast 0.5	7.0	0.3	2.0
8 = Na-Se 0.1	3.8	0.0	0.1
9 = Na-Se 0.3	3.2	0.1	0.1
10 = Na-Se 0.5	2.3	0.0	0.0

[1]Control (no Se added), L-SeMet 0.1 = L-selenomethionine-supplemented diet at 0.1 mg/kg, L-SeMet 0.3 = L-selenomethionine-supplemented diet at 0.3 mg/kg, L-SeMet 0.5 = L-selenomethionine-supplemented diet at 0.5 mg/kg, Se-yeast 0.1 = Se-enriched yeast-supplemented diet at 0.1 mg/kg, Se-yeast 0.3 = Se-enriched yeast-supplemented diet at 0.3 mg/kg, Se-yeast 0.5 = Se-enriched yeast-supplemented diet at 0.5 mg/kg, Na-Se 0.1 = sodium selenite-supplemented diet at 0.1 mg/kg, Na-Se 0.3 = sodium selenite-supplemented diet at 0.3 mg/kg, Na-Se 0.5 = sodium selenite-supplemented diet at 0.5 mg/kg.

$P = 0.008$; Table 4). A linear effect was found for L-selenomethionine and Se-enriched yeast, whereas for SS a plateau was reached after supplementing 0.3 mg/kg of Se. Comparable serum levels were found in laying hens fed either L-selenomethionine or Se-enriched yeast (Table 5).

The Se serum level increased by 213.05, 167.8, and 117.92 μg/L for L-selenomethionine, Se-enriched yeast, and SS per additional unit of the source supplemented in the diet, respectively.

The Se concentrations detected in the serum were reflected in the egg contents; a linear correlation between dosage of Se source and Se egg content was observed regardless of the Se source (Table 5). Highest and lowest mean levels were noticed when Se was added as L-selenomethionine and SS, respectively ($P < 0.001$; Table 4).

For the mean Se level in the egg contents, an increase of 777.44, 471.33, and 157.69 μg/kg was obtained per additional unit of L-selenomethionine, Se-enriched yeast, and SS supplement in the diet, respectively. The use of L-selenomethionine as Se source resulted in a significantly higher increase compared with the other 2 Se sources ($P < 0.001$), and a significantly higher increase

Table 4. The effect of source and inclusion level and their interaction on Se in serum (μg/L), glutathione peroxidase [GPx; U/g of hemoglobin (Hb)], and Se in egg contents (μg/L) after 56 d of treatment (n = 10/dietary treatment)[1]

Item	Se in serum (μg/L)	GPx (U/g of Hb)	Se in egg contents (μg/kg)
Treatment			
2 = L-SeMet 0.1	126.3^{c}	165.5^{ab}	299.9^{d}
3 = L-SeMet 0.3	195.4^{ab}	148.8^{abc}	472.9^{b}
4 = L-SeMet 0.5	238.1^{a}	122.8^{bc}	615.5^{a}
5 = Se-yeast 0.1	153.9^{bc}	84.5^{c}	242.3^{e}
6 = Se-yeast 0.3	196.4^{ab}	186.1^{ab}	374.7^{c}
7 = Se-yeast 0.5	226.5^{a}	222.1^{a}	480.9^{b}
8 = Na-Se 0.1	167.4^{bc}	131.9^{bc}	244.4^{e}
9 = Na-Se 0.3	191.6^{ab}	197.2^{ab}	288.0^{de}
10 = Na-Se 0.5	190.4^{ab}	185.1^{ab}	318.9^{cd}
SEM	5.0	6.9	13.21
Source			
L-SeMet	186.6	145.7	462.8^{a}
Se-yeast	192.3	164.2	366.0^{b}
Selenite	183.1	171.4	283.8^{c}
Supplementation level (mg/kg)			
0.1	149.2^{b}	127.3	262.2^{c}
0.3	194.5^{a}	177.4	378.5^{b}
0.5	218.3^{a}	176.7	471.8^{a}
P-value			
Source	NS	NS	<0.001
Level	<0.001	0.05	<0.001
Source × level	0.008	0.02	<0.001

[a–e]Means with the same letter are not significantly different from each other at $P \leq 0.05$.

[1]L-SeMet 0.1 = L-selenomethionine-supplemented diet at 0.1 mg/kg, L-SeMet 0.3 = L-selenomethionine-supplemented diet at 0.3 mg/kg, L-SeMet 0.5 = L-selenomethionine-supplemented diet at 0.5 mg/kg, Se-yeast 0.1 = Se-enriched yeast-supplemented diet at 0.1 mg/kg, Se-yeast 0.3 = Se-enriched yeast-supplemented diet at 0.3 mg/kg, Se-yeast 0.5 = Se-enriched yeast-supplemented diet at 0.5 mg/kg, Na-Se 0.1 = sodium selenite-supplemented diet at 0.1 mg/kg, Na-Se 0.3 = sodium selenite-supplemented diet at 0.3 mg/kg, Na-Se 0.5 = sodium selenite-supplemented diet at 0.5 mg/kg.

Table 5. Regression equations for measurements (Se concentrations in serum and in egg contents) having significant linear responses with progressive dietary added Se of different Se sources (n = 10/dietary treatment)

Response criteria and Se source	Equation	R^2	*P*-value
Se in serum (μg/L)			
L-SeMet[1]	Y = 102.8 + 279.5x	0.72	<0.001
Se-yeast[2]	Y = 137.8 + 181.5x	0.32	<0.001
Se in egg content (μg/kg)			
L-SeMet	Y = 226.1 + 789.1x	0.91	<0.001
Se-yeast	Y = 187.1 + 596.5x	0.87	<0.001
Selenite	Y = 227.9 + 186.1x	0.60	<0.001

[1]L-SeMet: L-selenomethionine.

[2]Se-yeast: Se-enriched yeast, x = Se concentration (mg/kg).

with Se-enriched yeast was observed in comparison to SS ($P < 0.001$; data not shown).

Comparing the levels at a dosage of 0.1 versus 0.5 mg/kg of the Se source, Se concentration in the egg contents increased only with a factor of 1.3 with SS, whereas levels were doubled with L-selenomethionine and Se-enriched yeast (Table 5).

There was a significant effect of Se source ($P < 0.001$) and of Se concentration ($P < 0.001$) on the obtained transfer factors [(concentration in the egg-content at d 56/calculated intake) × 100]. The highest transfer factors were obtained by supplementing the lowest level of Se independent of the Se source. By comparing the Se sources, L-SeMet resulted in the highest transfer factor, followed by Se-yeast and the lowest value was for the SS. Transfer factors of Se at a dosage of 0.1, 0.3, and 0.5 mg/kg were 43.31 ± 1.55, 42.27 ± 1.89, and 34.1 ± 1.28% for L-selenomethione; 36.59 ± 1.66, 33.26 ± 1.95, and 23.60 ± 0.74% for Se-enriched yeast; and 24.03 ± 0.91, 23.62 ± 0.84, and 15.88 ± 0.45% for SS, respectively (Figure 1).

GPx Concentrations in Serum

At the start of the trial, GPx concentrations were comparable between treatments (data not shown). After supplementing the diets for 56 d, no effect of dosage or source of Se was obtained for serum GPx levels. Increasing the dosage of SS or L-selenomethionine did not result in a significant increase of the serum GPx level. However, for the selenized yeast it appeared, as indicated by the interaction effect ($P = 0.022$), that a significant increase was observed by increasing the dosage from 0.1 to 0.3 mg/kg. In fact, this significant increase was due to the very low determined GPx level for laying hens fed the diet supplemented with selenized yeast at 0.1 mg/kg (Table 4).

DISCUSSION

The maximum level of Se for poultry in Europe is currently set at 0.5 mg/kg of complete feed (Commission Implementing Regulation No. 427/2013). Therefore, most studies examining the effect of a Se-enriched diet investigate diets containing no more than 0.5 mg/kg of Se. The higher levels in this study provide extra information about the response of laying hens and their egg Se content to a high dietary Se intake. In general, laying hens of the different dietary treatments were in good health, indicating no negative effect of the high dietary Se levels on laying hen performance. This is in accordance with previous studies indicating that even levels up to 6 mg/kg of anorganic as well as organic Se did not negatively affect body mass, feed intake, or egg production of laying hens (Payne et al., 2005; Utterback et al., 2005). A potential reason why performance is not improved by source or level of Se is the Se concentration of the basal diet, which was not free of Se as intended, but actually contained 0.25 mg/kg of Se. If a positive effect was seen in previous studies (Cantor and Scott, 1974), dietary Se level was deficient (<0.01 mg/kg), whereas in our trial the level was not deficient but close to the recommendation levels of the management guide (Lohmann Tierzucht, 2012) of layers but above the nutrient requirements of the NRC (1994).

The incidence of cracked eggs was comparable between treatments, which is in agreement with previous trials (Patton, 2000). In general, percentages were rather high during the trial due to the age of the laying hens at the start of the trial.

After supplementing the different Se sources for 8 wk, highest and lowest levels of Se in serum were found with L-selenomethionine and SS, respectively. Those Se concentrations detected in the serum reflected the supplemental Se in the diet and increased as the concentration of supplemental Se increased, regardless of Se source.

Selenium concentration in the egg contents also increased linearly as the Se supplementation increased. These results are in accordance with previous studies (Gajcevic et al., 2009; Yuan et al., 2011, 2013). At each supplementation level, organic Se, whether from L-selenomethionine or selenized yeast, resulted in a higher egg Se content than the inorganic source. By comparing both organic Se sources, a higher Se deposition in the egg by L-selenomethionine was found compared with the selenized-yeast-supplemented counterparts. These results indicate the source × level interaction. The differences in bioavailability between the supplemented Se sources can be explained by the active absorbance of

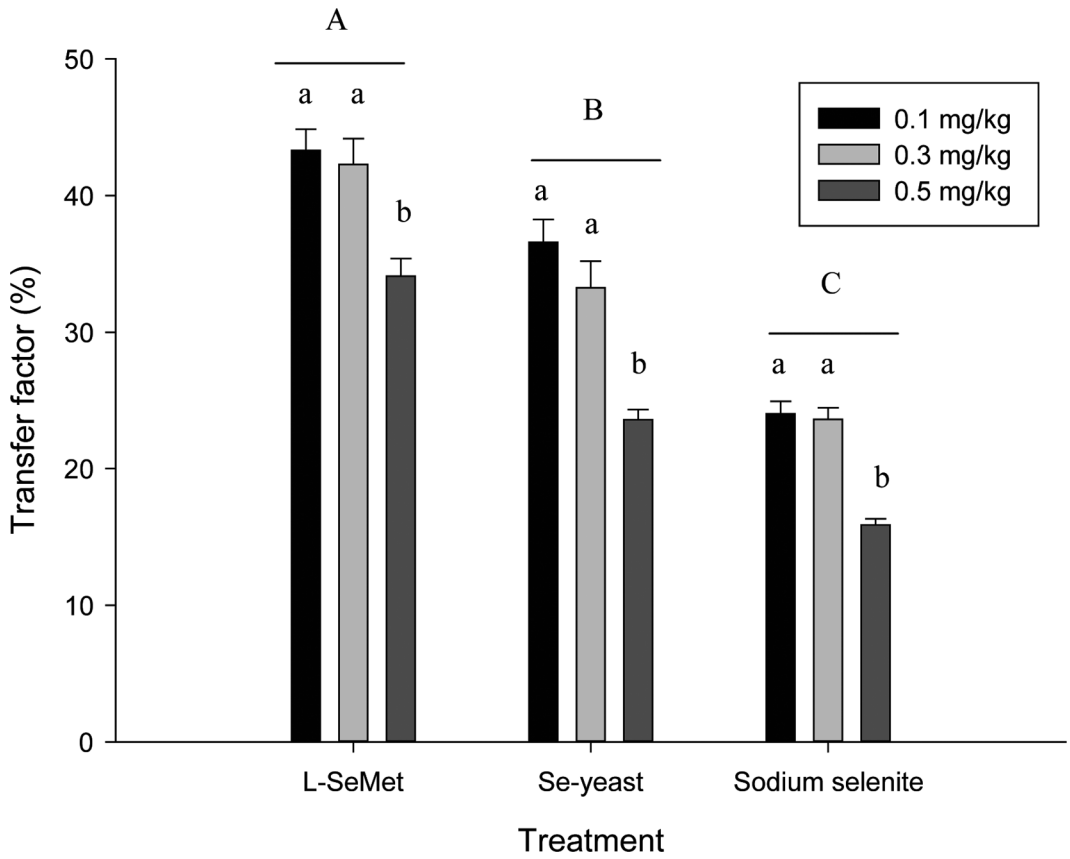

Figure 1. Transfer percentage of Se from the feed to the eggs for L-selenomethionine (L-SeMet), Se-enriched yeast (Se-yeast), and sodium selenite at 0.1, 0.3, and 0.5 mg/kg supplementation level for 56 d (n = 10/dietary treatment). Means of the different Se supplementation levels per Se source with the same lowercase letter (a,b) are not significantly different from each other at $P \leq 0.05$. Means of the different Se sources with the same uppercase letter (A–C) are not significantly different from each other at $P \leq 0.05$.

organic Se sources, whereas inorganic sources can only be passively absorbed (Payne et al., 2005). Furthermore, organic sources consist predominantly of selenomethionine and incorporation levels of selenomethionine and methionine into eggs are comparable (Latshaw and Biggert, 1981). Most of the Se present in selenized yeast is L-selenomethionine, but a significant amount of Se is also present in other forms. It is well established that selenomethionine is the major seleno-compound in selenized yeast. However, its proportion greatly varies (usually in a range 60–80%; Surai and Fisinin, 2014). Furthermore, the digestibility of selenized yeast could be a point of consideration, as the selenomethionine in selenized yeast is protein bound and the yeast protein needs to be digested before absorption. The L-selenomethionine is a free amino acid and not protein bound, so it does not require digestion before absorption. The difference in amount of Se as selenomethionine combined with the difference in digestibility might explain the higher transfer of Se from L-selenomethionine to eggs compared with selenized yeast.

If Se concentrations in eggs were calculated based on Se intake, the highest efficiency was shown with 0.1 mg/kg, followed by 0.3 mg/kg, and lowest with 0.5 mg/kg, regardless of the Se source. These data indicate that laying hens use Se more efficiently when the concentration of Se in the diet is low. These results are in agreement with those of Yoon et al. (2007), who found an inverse relationship between transfer of Se from diet to eggs relative to the concentration of Se supplemented. Transfer factors were also influenced by the dietary source of Se. This underscores the higher bioavailability of Se from organic sources compared with inorganic SS, with the highest bioavailability for L-selenomethionine as an organic source of Se.

Selenium is a component of GPx, a variable often used in bioefficacy studies (Payne et al., 2005; Yoon et al., 2007). In our trial, no effects of dosing or source of Se have been observed on GPx levels. This is in line with the results of Payne et al. (2005) and Yoon et al. (2007), who also found higher levels of Se in tissues or eggs by increasing the Se supplementation, but not of GPx. Therefore, no correlation between these variables could be found. A possible explanation for the absence of this effect could be the unexpected presence of Se in the control diet (no Se supplementation), meaning that the laying hens were not Se deficient. Burk et al. (2006) found that in a Se-deficient (deplete) status, biomarkers such as GPx can be useful, whereas in a steady Se status (replete) the biomarker GPx is above the plateau value and does not respond to increased supplementation.

However, enough Se reserves should be available for selenoprotein synthesis to effectively respond to envi-

ronmental challenges. Selenomethionine accumulated nonspecifically in muscle proteins can build Se reserves, which can be used in stress conditions when Se requirement is increased, but feed consumption usually decreased. Organic Se clearly shows an advantage in this regard and is therefore recommended for use in poultry and farm animal nutrition (Surai and Fisinin, 2014). A possible further advantage of bioavailable Se may be improvements in oxidative stability. Leeson et al. (2008) hypothesized that lower levels of GPx indicate better health status of the animal as these birds have a better oxidative stability. They found an increase of about 20% of GPx in blood and liver when rancid fat was used in the diets, implying that GPx may be used as a protection against damage caused by peroxides in the diet. These results indicate that under stress conditions, selenomethionine can be released and metabolized to GPx.

In conclusion, within the range of Se levels that were fed to the hens, a dose response effect of the different Se sources on Se concentrations in serum and eggs was observed in the present study. Supplementing 0.5 mg/kg of L-selenomethionine or selenized yeast instead of 0.1 mg/kg doubled the Se levels in the eggs, whereas supplementation with SS only increased the Se values by a factor of 1.3. Therefore, Se from organic sources was more bioavailable than the inorganic Se source as evidenced by blood and egg Se levels. The results also indicate that differences in bioavailability exist between organic Se sources as indicated by deposition of Se in egg contents. As the maximum supplementation level of organic Se in the European Union is currently limited to 0.2 mg of Se/kg of complete feed, the advantages of selenized yeast will be less pronounced when compared with inorganic Se sources. Alternative effective sources of organic Se such as L-selenomethionine, which show higher bioefficiency or higher transfer factors to the egg contents, could therefore provide additional benefit. In addition, it is hypothesized that these laying hens will have better antioxidant protection to cope with the stressful conditions of commercial poultry production.

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