The Effect of Dietary Selenium Source and Level on the Uptake of Selenium by Developing Chick Embryos[1]

N. D. Paton,[2] A. H. Cantor,[3] A. J. Pescatore, M. J. Ford, and C. A. Smith

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215

ABSTRACT We studied the effect of dietary source (organic or inorganic) and level of Se on the Se uptake of chick embryos. After receiving a low-Se diet for 16 wk, 126 Leghorn laying hens were randomly assigned to one of seven dietary treatments. Treatments consisted of feeding a low-Se basal diet alone or with one of three levels of added Se (0.1, 0.2, or 0.3 mg/kg Se) supplied by sodium selenite or Se-enriched yeast. Fertile eggs were collected after 33 d of feeding the experimental diets. Eggs were subjected to no incubation or incubation for 5, 10, 15, or 20 d. Non-incubated eggs were separated, and the yolk and albumen were assayed separately for Se. Incubated eggs were separated into the embryo and extra-embryonic portions, which were assayed separately for Se. Se concentrations of the yolk and albumen were significantly different among dietary treatments. Compared with eggs from hens fed sodium selenite, yolk and albumen Se concentrations were higher in eggs from hens fed Se yeast. Embryonic and extra-embryonic Se concentrations were higher in eggs from hens fed Se yeast than eggs from hens fed sodium selenite. The largest increase in embryonic Se concentration was observed during Days 10 to 15 of incubation. It was concluded that Se source and dietary inclusion level influenced the Se concentration of portions of developing embryonated eggs and that embryonic Se concentration changed during incubation.

(*Key words*: laying hen, chick embryo, selenium yeast, selenite)

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INTRODUCTION

Selenium is a required nutrient for the domestic chicken (*Gallus domesticus*) (National Research Council, 1994). Rotruck et al. (1973) reported that Se is an essential component of Se-dependent glutathione peroxidase (GSH-Px), an enzyme involved in cellular anti-oxidant protection. Depending on geographic location, many agricultural soils produce crops with limited Se content (National Research Council, 1983). As a result, most poultry feeds contain supplemental Se. Selenium supplementation of feeds is achieved using mainly inorganic sources of Se, such as sodium selenite (Na_2SeO_3). However, organic forms of Se such as high-Se yeast[4] (hereafter referred to as Se yeast) can also be used. The Se yeast is produced by growing a strain of yeast, *Saccharomyces cerevisiae*, in a high-Se medium. Selenomethionine, the Se analog of methionine, accounts for the largest portion of Se in Se yeast (Kelly and Power, 1995).

The effect of different dietary Se sources and inclusion levels on egg Se concentration has been described by many researchers (Cantor and Scott, 1974; Latshaw and Osman, 1974, 1975; Latshaw, 1975; Kääntee and Kurkela, 1980; Latshaw and Biggert, 1981; Kääntee et al., 1982; Martello and Latshaw, 1982; Moksnes and Norheim, 1982; Moksnes, 1983; Swanson et al., 1983; Laws et al., 1986; Robberecht et al., 1987; Swanson, 1987; Davis and Fear, 1996; Cantor et al., 2000). The research indicates that when organic forms of selenium are used (e.g., selenomethionine or Se yeast) and the dietary selenium concentration of the feed is increased from 0.1 mg to 0.5 mg Se /kg, the concentration of Se in the whole egg (fresh basis) increases from approximately 0.1 to 0.4 mg Se /kg. However, when inorganic sources of Se (e.g., sodium selenite) are used as the Se source, the incorporation of Se into the egg is not as great. If dietary Se concentration of hen feed is increased from 0.1 mg to 0.5 mg Se /kg using sodium selenite, the concentration of Se in the whole egg (fresh basis) increases from approximately 0.1 to 0.2 mg Se / kg. Dietary Se supplied in organic or inorganic form accumulates to a greater extent in the yolk of the egg than it does in the white. Increased dietary organic sources of Se elevate egg Se levels in the yolk and the white of the egg to a greater extent than do inorganic forms.

Selenium has been implicated as a factor affecting male fertility of poultry (Combs, 1994; Klecker et al., 1999). Se

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[2]Current address: Akey, Inc., CS 5002, Lewisburg, OH 45338.

[3]To whom correspondence should be addressed: acantor@uky.edu.

[4]Se yeast supplied as Sel-Plex, Alltech, Nicholasville, KY.

Abbreviation Key: GSH-Px = glutathione peroxidase.

supplementation increases the hatchability of fertile eggs from hens fed diets containing levels of Se below nutritional requirements (Poley et al., 1941; Cantor and Scott, 1974; Latshaw and Osman, 1974). As a result, Se has an important role in poultry fertility and embryonic development. However, there are currently no reports that quantify the movement of Se from the egg contents to the developing embryo. Because the developing embryo will eventually include almost all of the material inside the egg, it can be assumed that most of the Se present in the egg at oviposition will eventually reside in the tissues of the chick upon hatching. There may be differences in the amount and timing of Se absorption by the developing embryo. These differences may be affected by the source and level of dietary Se supplied to the hen and may affect the Se available to the developing embryo. The objective of the current study was to determine the effect of the maternal dietary Se source and level on the embryonic absorption of Se from the extra-embryonic material at five specific times during development.

MATERIALS AND METHODS

Birds and Diets

One hundred twenty-six Single Comb White Leghorn hens, 18 wk of age, were placed two birds per cage in laying cages (250 × 410 mm). The house temperature was maintained between 20 and 28 C. Approximately 20 lx of light was provided for 16 h per day. Water and feed were provided ad libitum. Each bird had access to one nipple drinker. Three contiguous cages shared one common feeding trough and made up the experimental unit. All procedures were approved beforehand by the University of Kentucky Institutional Animal Care and Use Committee.

All birds, irrespective of dietary treatment, were initially fed a low-Se depletion diet for 16 wk to deplete body reserves of Se and ensure uniformly low levels of Se in eggs. The ingredient and calculated nutrient compositions of the depletion diet are given in Table 1. The depletion diet and the experimental diets contained supplemental vitamin E at 20 IU / kg.

At 34 wk of age, three replicate groups of six hens (a total of 18 birds) were randomly assigned to each of seven dietary treatments. The seven experimental diets were fed ad libitum for 42 d. The dietary treatments consisted of feeding a low-Se basal diet alone or with three levels of added Se (0.1, 0.2, and 0.3 mg/kg Se) provided by sodium selenite or Se yeast.[5] The ingredient composition of the diets used for Se supplementation was the same as that of the depletion diet except for the Se supplementation. Se supplement was added to the depletion diet at 0.18% and was formulated to provide an additional 0.1, 0.2, or 0.3 mg/kg Se from sodium selenite or a commercial Se yeast preparation. The Se content of the depletion diet and the seven experimental diets were determined analytically and is presented in Table 2. Feed intake of the experimental diets was measured weekly. Body weight was measured at the start and end of the experimental period. Total egg production for each replicate was recorded daily.

Fertile Egg Collection

All the hens were artificially inseminated on Days 30, 31, and 38 of the experimental period, according to the method described by Burrows and Quinn (1937). Semen was collected from trained roosters fed a diet containing supplemental Se and vitamin E. During Days 33 through 42 of the experimental period, 1,179 eggs were collected and stored in an egg cooler (15 C) in preparation for incubation. There were five incubation treatments based on length of incubation. Eggs were incubated for 0 (not incubated), 5, 10, 15, or 20 d. At least six eggs from each replicate group of hens were randomly assigned to each incubation period, with at least 21 eggs from each replicate being randomly assigned to the 5-d incubation period. The Day 0 (non-incubated) eggs were set aside, and the remaining eggs were incubated at 37.5 C and a relative humidity of 55 to 60 %.[6] Weights of eggs collected for incubation were recorded daily. Egg weights were recorded again after removal from the incubator or, in the case of eggs not incubated, after removal from storage.

Egg and Embryo Se Determination

Eggs that were not incubated were broken out and the albumen and yolks were separated, pooled within a replicate, and weighed. Embryos in eggs that were incubated were killed by chilling at 4 C for 4 h (Federation of Animal Science Societies, 1999). The incubated eggs were broken out; the developing embryo was separated from the remaining egg contents, and the embryo and the extra-embryonic material were collected. The embryonic portion consisted of only the physical embryo, whereas the extra-embryonic portion consisted of the remaining yolk and yolk sac membrane, albumen, and amniotic fluid, and membrane. Within a replicate, the embryos were pooled, weighed, and homogenized using an electric hand blender. The extra-embryonic egg material was processed in a similar manner. Any eggs that were infertile or contained embryos that had suffered early embryonic death were discarded.

Samples of the homogenate of the yolk and white of non-incubated eggs, and embryonic and extra-embryonic portions of incubated eggs, were subjected to Se analysis using the fluorometric method of Olson et al. (1975) as modified by Cantor and Tarino (1982). With this method, aliquots of all samples (homogenates of albumen, yolk, embryo, and extra-embryonic material) were weighed into digestion tubes and subjected to Se quantification. Total egg Se for each period was calculated from weights

[5]Sel-Plex, Alltech, Inc., Nicholasville, KY.
[6]Humidare Incubator Model 200, Humidare Incubator Co., New Madison OH.

TABLE 1. Ingredient and nutrient composition of the depletion diet

Ingredient	% of Diet	Nutrient[1]	Calculated analysis
Corn	58.36	AME_n, kcal/kg	2,866
Soybean meal (48%)	27.97	Protein, %	18.5
Corn oil	2.48	Calcium, %	3.9
Salt	0.46	Available P, %	0.3
Limestone	6.43	Fat, %	4.75
Oyster shell	3.00	Linoleic acid, %	2.89
Dicalcium phosphate	1.04	Methionine, %	0.47
DL-Methionine	0.17	TSAA, %	0.78
Vitamin-mineral mix[2]	0.10	Lysine, %	0.99

[1]Values reported are on an as-is basis.

[2]Supplied per kilogram of diet: 10,000 IU vitamin A, 2,000 IU vitamin D_3, 20 IU vitamin E, 2 mg vitamin K, 14 μg vitamin B_{12}, 6 mg riboflavin, 8 mg pantothenic acid, 30 mg niacin, 150 mg choline, 2 mg pyridoxine, 1 mg thiamin, 1 mg folic acid, 60 μg biotin, 75 mg iron, 65 mg zinc, 60 mg manganese, 10 mg copper and 400 μg iodine. No Se was provided in the vitamin-mineral mix.

and Se concentrations of egg yolk and white (Day 0) or of embryonic and extra-embryonic material (Days 5, 10, 15, and 20).

Statistical Analyses

Using the general linear models procedure of SAS software (SAS Institute, 1999), data were subjected to ANOVA procedures appropriate for a completely randomized design (Snedecor and Cochran, 1989). There was a factorial arrangement of treatments using seven diets and five incubation periods. An orthogonal set of contrasts (Snedecor and Cochran, 1989) was used to identify dietary treatment differences resulting from the addition of Se (vs. no Se addition), Se source (Se yeast or selenite), Se level (0.1, 0.2, or 0.3 mg/kg additional Se), and whether the response to additional levels of both types of Se was linear or quadratic in nature. Source by linear or source by quadratic interaction contrasts were performed to test whether the linear or quadratic responses were similar for both sources of Se. A set of orthogonal polynomial contrasts was used to describe embryonic and extra-embryonic Se content of the eggs at different periods of incubation.

RESULTS AND DISCUSSION

Production Performance

Average feed intake (90 g/hen per d) and body weight change from the start to the end of the experimental period (79 g) were not significantly ($P < 0.05$) affected by dietary treatments. During the experimental period, hen-day egg production (93.6%) and average egg weight (53.4 g) were also not significantly ($P < 0.05$) affected by dietary treatments.

Egg Se Content

Total egg selenium content for eggs within each of the seven dietary treatments was not significantly affected by incubation time (Table 3). The total egg Se content should not change throughout the incubation period, because this change would indicate a net loss or gain of the element to or from an outside source and would jeopardize interpretation of the results. There were significant differences in total egg Se amount among dietary treatments. The contrasts performed indicated that eggs from hens given Se-supplemented diets contained more Se than did those from hens fed the basal diet. The significant source of Se contrast indicated that eggs from hens fed the Se yeast diets contained significantly more Se than did eggs from hens fed the selenite diets. There was a positive linear response to increasing Se supplementation, but this response was dissimilar for the two Se sources. Increases in Se whole egg contents were greater when dietary Se source of hens was Se yeast than when Se was provided by sodium selenite.

Data quantifying the effect of dietary treatment on the Se concentration of egg yolk, albumen, and whole egg

TABLE 2. Selenium concentration of the depletion diet and experimental feeds[1]

Diet	Assayed Se concentration,[2] mg/kg ± SD
Depletion diet	0.059 ± 0.003
Basal diet, no added Se	0.057 ± 0.001
0.1 mg/kg Se as Na_2SeO_3	0.165 ± 0.009
0.2 mg/kg Se as Na_2SeO_3	0.226 ± 0.020
0.3 mg/kg Se as Na_2SeO_3	0.340 ± 0.009
0.1 mg/kg Se as Se yeast	0.156 ± 0.014
0.2 mg/kg Se as Se yeast	0.227 ± 0.004
0.3 mg/kg Se as Se yeast	0.305 ± 0.001

[1]Values are means ± SD for three assays. The depletion diet (18 to 34 wk) and basal diet (34 to 40 wk) used the same formula.

[2]Values are reported on an as-is basis.

TABLE 3. Effect of diet and incubation on total selenium content of eggs[1]

Diet	Day of incubation[2]					
	0	5	10	15	20	SEM
	Se, μg[3]					
Basal, no added Se (Basal)	2.6	2.0	2.3	2.4	2.6	0.21
0.1 mg/kg Se as Na_2SeO_3	6.6	5.7	5.5	5.5	5.1	0.54
0.2 mg/kg Se as Na_2SeO_3	7.1	5.9	6.5	5.7	5.4	0.59
0.3 mg/kg Se as Na_2SeO_3	6.9	6.8	7.0	5.8	5.8	0.42
0.1 mg/kg Se as Se yeast	7.1	6.2	6.8	6.1	6.6	0.41
0.2 mg/kg Se as Se yeast	8.7	8.4	8.6	7.0	7.3	0.63
0.3 mg/kg Se as Se yeast	11.2	10.8	11.5	9.8	9.5	0.68
SEM	0.49	0.54	0.36	0.39	0.27	
	Orthogonal contrasts					
Se supplementation[4]	**	**	**	**	**	
Source of Se[5]	**	**	**	**	**	
Linear response to level of Se	**	**	**	**	**	
Quadratic response to level of Se	NS	NS	NS	NS	NS	
Source × linear interaction	**	**	**	**	**	
Source × quadratic interaction	NS	NS	NS	NS	NS	

[1]For each incubation period, data are means from all eggs collected from three groups of six birds.

[2]Within rows data were not significantly different ($P \leq 0.05$).

[3]Calculated from weights and Se concentrations of egg yolk and white (Day 0) or of embryonic and extra-embryonic material.

[4]Basal diet vs. Se-supplemented diets.

[5]0.1, 0.2, and 0.3 mg/kg Se as Na_2SeO_3 as 0.1, 0.2, and 0.3 mg/kg Se as Se yeast.

**$P \leq 0.01$.

for eggs that were not incubated are presented in Table 4. Data collected in this trial are in agreement with earlier work describing the Se content of eggs as affected by the diet of the hen (Cantor and Scott, 1974; Latshaw and Osman, 1974, 1975; Latshaw, 1975; Kääntee and Kurkela, 1980; Latshaw and Biggert, 1981; Martello and Latshaw, 1982; Moksnes and Norheim, 1982; Kääntee et al. , 1982; Moksnes, 1983; Swanson et al., 1983; Laws et al., 1986; Swanson, 1987; Robberecht et al., 1987; Davis and Fear, 1996; Cantor et al., 2000). Whole egg Se is directly affected by the level of Se in the diet of the maternal hen. Se supplementation in any form elevated the Se concentration of all the egg portions, as indicated by the orthogonal contrasts presented in Table 4. The source of dietary Se also affected egg Se concentration in all egg components. The addition of Se as Se yeast (vs. selenite) resulted in egg portions with greater concentrations of Se. For all components, increasing levels of supplemental Se (irrespective of source) produced significant linear increases in egg Se concentration; however, the linear responses were different for the different sources of Se in all egg components. Feeding increasing levels of organic sources

TABLE 4. Effect of diet on selenium concentration of egg yolk, egg white, and whole egg[1]

Diet	Egg yolk	Egg white	Whole egg contents[2]
	Se, $\mu g/g$		
Basal, no added Se	0.10	0.04	0.06
0.1 mg/kg Se as Na_2SeO_3	0.33	0.07	0.14
0.2 mg/kg Se as Na_2SeO_3	0.37	0.07	0.16
0.3 mg/kg Se as Na_2SeO_3	0.38	0.07	0.16
0.1 mg/kg Se as Se yeast	0.32	0.08	0.15
0.2 mg/kg Se as Se yeast	0.42	0.13	0.22
0.3 mg/kg Se as Se yeast	0.48	0.15	0.25
SEM	0.012	0.013	0.011
	Orthogonal contrasts		
Se supplementation[3]	**	**	**
Source of Se[4]	**	**	**
Linear response to level of Se	**	*	**
Quadratic response to level of Se	NS	NS	NS
Source × linear interaction	**	*	**
Source × quadratic interaction	NS	NS	NS

[1]Data presented are means for eggs from three groups of six hens, fresh basis.

[2]Value calculated from weights and Se concentrations of yolk and white.

[3]Basal diet vs. Se-supplemented diets.

[4]0.1, 0.2, and 0.3 mg/kg Se as Na_2SeO_3 vs. 0.1, 0.2, and 0.3 mg/kg Se as Se yeast.

*$P \leq 0.05$; **$P \leq 0.01$.

TABLE 5. Main effect of length of incubation on weights of embryos and extra-embryonic material[1]

Incubation period, d	Embryo, g	Extra-embryonic material, g
5	0.1	44.9
10	1.9	42.0
15	10.0	33.3
20	36.2	5.2
SEM	0.18	0.31
	Orthogonal polynomial contrasts	
Linear	**	**
Quadratic	**	**

[1]Within each incubation period data presented are means for eggs from each of 21 groups of six birds.

**$P \leq 0.01$.

of Se resulted in greater rates of Se deposition in yolks and whites compared with feeding selenite. A possible reason for elevated concentrations of Se in the yolk, white, and egg contents due to providing dietary Se as Se yeast is that the hen has additional metabolic pathways by which to transfer Se into the egg. For example, higher egg albumen Se levels in eggs from hens fed Se yeast may be due to incorporation of greater amounts of Se as selenomethionine. The selenomethionine could substitute for methionine during albumen synthesis, thereby providing additional Se.

Embryo and Extra-Embryonic Selenium Concentration

The main effect of dietary treatment on embryo and extra-embryonic weights was not significant. Therefore, Se concentration differences among dietary treatments were not due to differences in weight among embryos or extra-embryonic material. As expected, the main effects of length of incubation were significant differences in embryo and extra-embryonic weights (Table 5). As incubation proceeded, embryonic weights increased, and extra-embryonic weights decreased in a quadratic relationship with increasing length of incubation.

The main effects of dietary and incubation treatments on embryo and extra-embryonic Se concentration were significant (Table 6). Significant differences among the values for the seven dietary treatments were observed within each period of incubation. As evidenced by the significant Se supplementation contrast in every period of incubation, embryos from the basal dietary treatment had Se concentrations that were significantly lower than embryos from other treatments. Se source had an impact on the embryo Se concentration in every incubation period. Embryos from Se yeast treatments had higher Se concentrations than those from selenite treatments. In all cases, the response to additional Se was linear. However the linear response was not the same for the two types of Se.

Within all incubation periods, the linear increase in embryonic Se concentration due to the addition of greater levels of selenite was more gradual than the linear response to increasing levels of supplemental Se provided by Se yeast. The fact that dietary treatment affected the Se concentration in the developing embryos is not surprising, considering the previously cited reports showing the effect of dietary Se on egg Se concentration. On Days 10, 15, and 20, the embryo Se concentrations in embryos from the 0.1 mg/kg Se yeast treatment (0.9, 0.14, and 0.14 μg/g Se respectively) were higher than those in embryos from the 0.3 mg/kg selenite treatment (0.08, 0.08, and 0.12 μg/g Se). In this case, more Se was being delivered to the embryo from a diet containing a third of the concentration of added Se. Under commercial conditions, this may be highly advantageous, considering that feeds are not necessarily homogeneously mixed and portions of the feed may fail to deliver sufficient amounts of the mineral. The Se content of the hen's diet has been shown to influence the amount of Se concentration and the activity of GSH-Px in the newly hatched chick (Combs and Scott, 1979; Hassan, 1986; Surai, 2000). These data suggest that this phenomenon is also observed during incubation.

In all dietary treatments with supplemental selenium, there was a significant linear increase in embryo Se concentration as incubation proceeded. The largest increase was observed between Days 10 and 15, when average embryo Se concentration increased 0.05 μg/g. This observation may be related to reports by Surai (1999) and Surai et al. (1997), which noted that the activity of GSH-Px in the liver of the developing chick embryo rises rapidly during Days 10 to 15 of incubation. An increase in the activity of this enzyme in the chick would require additional Se, which results in increased Se uptake by the embryo as was demonstrated by Omaye and Tappel (1974), Combs and Scott (1979), Hassan (1986), and Surai (2000). Wilson et al. (1992) reported a linear increase in levels of GSH-Px activity in the chick liver between Days 8 and 18 of embryonic development. The activity of GSH-Px also increased in the brain of developing chick embryos during Days 10 to 18 of development (Wilson et al., 1992). This increase was associated with the development of glial cells in the brain after Day 10. Wilson et al. (1992) also noted an increase in the activities of catalase and superoxide dismutase (SOD) in the liver of developing embryos between Days 10 and 18 of embryonic development. Surai et al. (1996) and Noble et al. (1993) reported an increase in the concentration of α-tocopherol in the liver of developing chick embryos after Day 13. These published reports suggest that metabolic processes during this stage of embryonic development are susceptible to oxidative attack, and the embryo ensures that sufficient antioxidant protection is available. If this were the case, then increases in the absorption of Se by the embryo during this stage of development would be necessary. Data presented in Table 6 support this hypothesis.

The data tabulated in Table 6 show that the effect of incubation on extra-embryonic Se concentration from 5 to 15 d of incubation was small. However there was a large increase in extra-embryonic Se concentration between Days 15 and 20. Over the entire incubation period, the increases in extra-embryonic Se concentration were

TABLE 6. Effect of diet and incubation on embryonic and extra-embryonic selenium concentration of incubated eggs[1]

Diet	Embryonic Se Day of incubation				SEM	Orthogonal polynomial contrasts[2]		Extra-embryonic Se Day of inbuation				SEM	Orthogonal polynomial contrasts	
	5	10	15	20		L	Q	5	10	15	20		L	Q
	Se, μg/g							Se, μg/g						
Basal, no added Se	0.06	0.06	0.08	0.06	0.001	NS	**	0.04	0.05	0.05	0.07	0.003	**	NS
0.1 mg/kg Se as Na_2SeO_3	0.08	0.08	0.11	0.10	0.002	**	*	0.12	0.13	0.13	0.18	0.005	**	**
0.2 mg/kg Se as Na_2SeO_3	0.09	0.08	0.11	0.10	0.004	**	NS	0.13	0.15	0.14	0.23	0.011	**	*
0.3 mg/kg Se as Na_2SeO_3	0.09	0.08	0.12	0.12	0.003	**	NS	0.15	0.17	0.14	0.28	0.006	**	**
0.1 mg/kg Se as Se yeast	0.09	0.09	0.14	0.14	0.005	**	NS	0.13	0.16	0.14	0.20	0.009	**	*
0.2 mg/kg Se as Se yeast	0.10	0.11	0.16	0.14	0.007	**	NS	0.19	0.20	0.17	0.32	0.014	**	**
0.3 mg/kg Se as Se yeast	0.12	0.11	0.18	0.19	0.005	**	NS	0.24	0.27	0.23	0.36	0.015	**	**
SEM	0.001	0.003	0.003	0.007				0.012	0.008	0.010	0.008			
	Orthogonal contrasts													
Se supplementation[3]	**	**	**	**				**	**	**	**			
Source of Se[4]	**	**	**	**				**	**	**	**			
Linear response	**	*	**	**				**	**	**	**			
Quadratic response	NS	NS	NS	NS				NS	NS	NS	*			
Source × linear	**	*	**	*				**	**	**	**			
Source × quadratic	NS	NS	NS	NS				NS	NS	NS	*			

[1]Within each incubation period, data presented are means for all eggs from three groups of six birds, fresh basis.

[2]L = linear, Q = quadratic.

[3]Basal diet vs. Se-supplemented diets.

[4]0.1, 0.2, and 0.3 mg/kg Se as Na_2SeO_3 vs. 0.1, 0.2, and 0.3 mg/kg Se as Se yeast.

*$P \leq 0.05$; **$P \leq 0.01$.

quadratic for all dietary treatments containing supplemental selenium. Although extra-embryonic concentrations of Se were highest on Day 20 of incubation, it should be noted that the actual amount of Se is small. Based on the average weight of the extra-embryonic material on Day 20 (Table 5), the extra-embryonic material from the basal treatment would only account for 0.4 μg of Se, whereas the extra-embryonic material from the 0.3 mg/kg yeast treatment would account for 1.9 μg of Se. The embryo would have absorbed a large amount of the extra-embryonic material at this stage, leaving only a small amount that is concentrated with Se.

Surai (1999) reported that there were large increases in activity of GSH-Px in the yolk sac membrane after Day 10 of embryonic development, which would require extra Se. Because the yolk sac membrane is part of the portion analyzed with the extra-embryonic material the movement of Se from other portions of the extra-embryonic material to the yolk sac membrane would go undetected in this study.

As shown in Table 6, there were significant differences among dietary treatment means for the extra-embryonic Se concentration in every period of incubation. Feeding the basal diet resulted in the lowest extra-embryonic Se concentration. The contrast for Se supplementation was highly significant in every period. Source of Se also had a significant effect on the extra-embryonic concentration of Se. Material from Se yeast treatments had a higher Se concentration than those from selenite treatments. The response to additional supplementation of Se was linear for both sources of Se. There was a significant difference between the linear responses of the two Se sources. Increases in dietary Se from feeding greater amounts of Se yeast elevated extra-embryonic Se concentration to a greater extent than did similar dietary Se additions using sodium selenite. The higher extra-embryonic Se concentration observed in the Se yeast dietary treatments was not an indication of reduced or delayed transfer of Se to the embryo but rather reflected an increased Se status in general, as indicated by total egg Se (Table 3).

This work clearly demonstrates that the source and level of Se has a large influence on the amount of Se transferred to the developing embryo. It would also appear that the embryo absorbs greater amounts of Se during Days 10 to 15 of incubation than during other periods. This may reflect a changing requirement for the mineral during incubation that could be related to physiological and developmental processes that occur while the embryo is maturing.

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