THE EFFECT OF SELENIUM YEAST IN THE HEN'S DIET ON TRANSFER OF SELENIUM TO THE EGG AND THE DEVELOPING EMBRYO

DJELOVANJE SELENSKOG KVASCA U HRANI KOKOŠI NA PRIJENOS SELENA U JAJE I EMBRIJ U RAZVOJU

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SUMMARY

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 which could be related to physiological and developmental processes that occur while the embryo is maturing. Improving the transfer of Se from the hen's diet by using an Se yeast instead of inorganic sodium selenite is a useful strategy to improve the nutritional status of the embryo as well as that of the newly hatched chick.

Key words: selen, source, embryo, Se yeast, sodium selenite

INTRODUCTION

Selenium (Se) was reported to be an essential dietary nutrient over 45 years ago, when Schwarz and Foltz (1957) demonstrated that Se was the elusive Factor 3 that prevented liver necrosis in rats. In the same year there were two reports showing that Se was the factor that prevented exudative diathesis in chicks (Parterson et al., 1957; Schwarz et al., 1957). Ten years later, Scott et al. (1967) observed that Se could prevent gizzard and cardiac myopathies in young turkey poults. Many symptoms of Se deficiency can be alleviated by vitamin E. The effectiveness of vitamin E, however, depends on the degree of the Se deficiency. In the case of a very severe Se deficiency, the chick develops pancreatic fibrosis (Thompson and Scott, 1970), a condition that reduces secretions of pancreatic digestive enzymes. This, in turn, reduces absorption of a number of nutrients, including fat and fat-soluble vitamins. Therefore, dietary vitamin E is ineffective in preventing pancreatic fibrosis.

While most Se deficiencies are seen in very young birds, a lack of dietary Se can also affect older birds. While Se deficiency has been shown to cause depressed egg production in both chickens and turkeys, it has a much greater impact on hatchability (Cantor and Scott, 1974; Cantor et al., 1978; Latshaw and Osman, 1974). When turkey breeder hens were given diets deficient in both Se and vitamin E, the poults that did hatch had classical signs of gizzard myopathy (Cantor et al., 1978). It is interesting to note that in a very early

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study focused on Se toxicity (Poley et al., 1941) Se supplementation of the hen's diet improved hatchability. Selenium has also been implicated as a factor affecting male fertility of poultry (Combs, 1994; Klecker et al., 1999).

The National Research Council (1994) in the USA lists the Se requirements for turkeys, ducks, broiler chickens and laying hens at 0.2, 0.2, 0.15 and 0.06 mg/kg diet. However, there have been numerous observations of benefits from using higher Se levels in animal diets in both practical poultry production as well as in research trials. In the USA, the Food and Drug Administration permits poultry feed manufacturers to include up to 0.3 mg Se/kg feed.

Meeting the dietary requirement for Se depends on a number of factors, including 1) the amount of naturally occurring Se in the feed ingredients; 2) the amount of supplemental Se used; 3) the biological availability of Se in the ingredients and 4) in the case of the very young chick, the carryover of Se from the hen. There has been considerable concern about meeting the Se needs of poultry and livestock in the USA and other countries. The reason for this is that in various geographic locations, agricultural soils produce crops with limited Se content (National Research Council, 1983). In a survey of Se levels in feed ingredients in the USA, we encountered samples of corn containing only 0.006 mg Se/kg and of soybean meal having only 0.06 mg Se/kg (Cantor, 1997). Poultry diets based on such ingredients would clearly not meet the Se requirement with supplemental sources.

The biological availability of the Se in an ingredient can vary greatly, depending on the ingredient or compound. For example, good availability values (60 to 90%) for feedstuffs of plant origin and poor availability values (<25%) for feedstuffs of animal origin were obtained in studies using sodium selenite as a standard and prevention of exudative diathesis in chicks as a criterion (Cantor et al., 1975b). However, it should be noted that availability values for the same ingredient or compound can vary, depending on the criteria used and the conditions of the assay. For example, the availability of selenomethionine was much higher preventing pancreatic fibrosis than for preventing exudative diathesis in chick (Cantor et al., 1975a, b). Basically, when we look at availability of a

nutrient, we are concerned with such factors as the degree of absorption, retention, and utilization for biological functions in the animal.
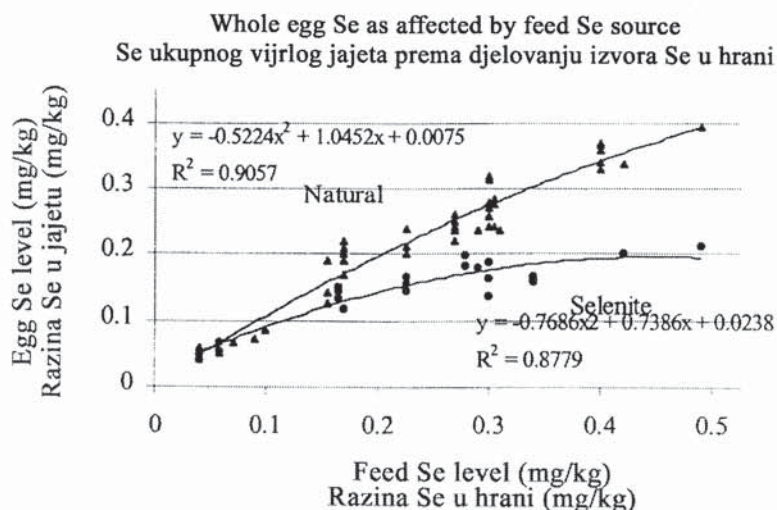
SELENIUM YEAST AS A SELENIUM SUPPLEMENT

For many years, the inorganic salts, sodium selenite and sodium selenate, were the only Se supplements approved for use in the USA. Selenium yeast (Sel-Plex, Alltech Biotechnology, Nicholasville, KY, USA) was approved as a supplemental form in 2000. Selenium yeast is produced by growing yeast 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 a number of researchers (Cantor and Scott, 1974; Latshaw and Osman, 1974, 1975; Latshaw, 1975; Määntee and Kurkela, 1980; Latshaw and Biggert, 1981; Martello and Latshaw, 1982; Moksnes and Norheim, 1982; Määntee 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). These research reports indicated that when organic forms of Se are used (e.g., selenomethionine or Se yeast) and the dietary Se 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 (Figure 1). 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 the hen's 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 either organic or inorganic form accumulates to a greater extent in the yolk of the egg than it does in the white. Compared with inorganic sources, dietary organic sources of Se are more effective in elevating Se levels in the yolk and the white of the egg.

Figure 1.   The effect of feeding naturally occurring (organic) and selenite Se on whole egg Se, fresh basis (From Paton, 2001).

Slika 1.   Djelovanje prirodne (organske) hranidbe i selenita Se na Se čitavog jajeta, na svježu osnovu (Prema Paton, 2001).

Whole egg Se as affected by feed Se source
Se ukupnog vijrlog jajeta prema djelovanju izvora Se u hrani

$y = -0.5224x^2 + 1.0452x + 0.0075$
$R^2 = 0.9057$
Natural

Selenite
$y = -0.7686x2 + 0.7386x + 0.0238$
$R^2 = 0.8779$

Egg Se level (mg/kg)
Razina Se u jajetu (mg/kg)

Feed Se level (mg/kg)
Razina Se u hrani (mg/kg)

In one of our early studies with Se yeast in laying hen diets, a low-Se diet was alone or with 0.3 mg Se/kg diet as either sodium selenite or Se yeast to commercial White Leghorn hens for 6 weeks (Cantor et al., 2000). While both forms of Se elevated egg Se, the increase with Se yeast was 24 to 31% greater than that obtained with selenite (Table 1). This suggested that Se yeast could be used to produce nutritionally enhanced eggs for human consumption and to improve the Se status of the embryo and the newly hatched chick.

Table 1.   Effect of supplemental Se as sodium selenite and Se yeast in laying hen diets on egg Se concentration and content[1]

Tablica 1. Djelovanje dodavanja Se kao natrijevog selenita i Se kvasca u obroke kokoši nesilica na koncentraciju i sadržaj Se u jajetu

	Egg Se concentration, g/g, fresh basis[2] Koncentracija Se u jajetu g/g, svježa osnova		Egg Se content, g[2]-Sadržaj Se u jajetu	
Diet[3] - Obrok	3 weeks 3 tjedna	6 weeks 6 tjedana	3 weeks 3 tjedna	6 weeks 6 tjedana
Basal - Osnovni	0.047	0.059	2.57	3.28
+0.3 as sodium selenite	0.182	0.197	9.89	10.9
+0.3 mg Se/kg Se as Se yeast	0.235	0.235	13.0	13.5
Standard Error of the Mean	0.004	0.006	0.22	0.42

[1] From Cantor et al. (2000).

[2] Each value is the mean of eight pooled samples of four eggs per replicate. Eggs were sampled after 3 and 6 weeks of feeding experimental diets - Svaka vrijednost je prosjek od 8 spojenih uzoraka od 4 jajeta po ponavljanju. Jaja su skupljana nakon 3 i 6 tjedna hranjenja pokusnim obrocima

[3] Each diet was fed to eight replicate groups of six hens - Svaki je obrok davan u 8 spojenih skupina od 6 kokoši

EMBRYONIC UPTAKE OF Se

To verify improvements in the Se status of embryos, a study was initiated to quantify the movement of Se from the egg contents to the developing embryo (Paton et al., 2002). Because the developing embryo includes almost all of the material inside the egg, it was assumed that most of the Se present in the egg at oviposition would eventually reside in the tissues of the chick upon hatching. However, we were interested in observing if the source or the level of dietary Se in the hen's diet could cause differences in the amount and timing of Se absorption by the developing embryo. Therefore, we examined 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. A total of 126 Single Comb White Leghorn hens were initially fed a low-Se depletion diet for 16 weeks to deplete body reserves of Se and ensure uniformly low levels of Se in eggs. Then each of seven experimental treatments were administered to six replicate forups of 3 hens diets for 42 days. The dietary treatments consisted of feeding a low-Se basal diet (0.057 mg Se/kg) 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 (Sel-Plex). Following artificial insemination of hens after feeding the experimental diets for 30 days, the eggs that were collected were incubated for 0 (not incubated), 5, 10, 15 or 20 days. 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 day 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%.

Eggs which 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. The incubated eggs were broken out and the contents were separated into the embryo and the extra-embryonic material. The embryo portion consisted of only the physical embryo, while the extra-embryonic portion consisted of the remaining yolk and yolk sac membrane, albumen, and the amniotic fluid and membrane. Pooled homogenized samples of yolk and white of non-incubated eggs, and of embryonic and extra-embryonic portions of incubated eggs, were subjected to Se analysis.

Average feed intake (90 g/hen/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.

The effect of dietary treatments on the Se concentration of egg yolk, albumen and whole egg for eggs that were not incubated are presented in Table 2. These results agree with the previously cited studies on the influence of the hen's diet on the Se content of eggs. All six Se supplements significantly increased the Se concentrations in yolk, white and whole egg contents. Feeding increasing levels of organic Se resulted in greater rates of Se deposition in yolk, white, and egg contents, compared with selenite. A possible reason for elevated concentrations of Se in the egg obtained with Se yeast is that the hen has additional metabolic pathways by which to transfer Se into the egg, Selenomethionine can substitute for methionine during albumen and yolk protein synthesis, thereby providing additional Se.

Selenium supplementation of the hens' diets resulted in significant linear increases in embryonic and extra-embryonic Se concentration at 5, 10, 15 and 20 days of incubation. Figure 2 shows embryonic Se data at 15 days, at which time the concentration of Se in the embryo was maximized. Embryos from Se yeast treatments had higher Se concentrations than those from selenite treatments. Embryo Se concentrations in embryos from the 0.1 mg/kg Se yeast treatment were higher than those in embryos from the 0.3 mg/kg selenite treatment. Thus, 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.

Figure 2. Effect of supplementing a low-Se diet with Se as selenite or Se yeast on embryonic Se concentrations after 15 days of incubation. Significant (P<0.05) increases resulted from Se supplementation in either form vs. the basal diet and from the use of Se yeast vs. selenite. Adapted from Paton et al. (2002).

Slika 2. Djelovanje dodavanja Se u obliku selenita ili selenskog kvasca obrocima s niskom Se na koncentracije embrijskog Se nakon 15 dana inkubacije. Značajni (P<0,05) porasti posljedica su dodavanja selena u bilo kojem obliku u usporedbi s temeljnim obrocima te upotrebe Se kvasca u usporedbi sa selenitom. Prema Paton i sur., (2002)
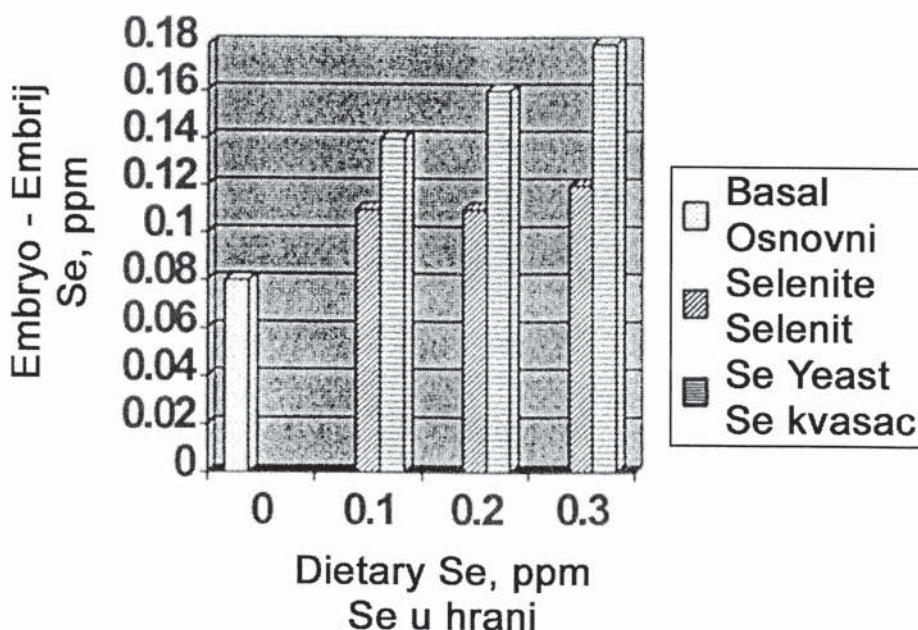
In all dietary treatments with supplemental Se, there was a significant linear increase in embryo Se concentration as incubation proceeded (data not shown). The largest increase was observed between day 10 and 15, when average embryo Se concentration increased 0.05 g/g. This observation may be related to reports by Surai (1999) and Surai et al. (1997), which noted that the activity of Se-dependent glutathione peroxidase 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 glutathione peroxidase activity in the chick liver between days 8 and 18 of embryonic development. The activity of glutathione peroxidase 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 day 10 and 18 of embryonic development. Surai et al. (1996) and Noble et al. (1993) reported an increase in the concentration of -tocopherol in the liver of developing chick embryos after day 13. These published reports suggest that metabolic processes during this stage of embryonic development make the embryo susceptible to oxidative attack. Thus, increased absorption of Se and vitamin E by the embryo during this stage of development would be necessary to provide antioxidant protection. The results shown in Table 2 support this hypothesis.

Table 2. Effect of diet on Se concentration of egg yolk, egg white and whole egg1
Tablica 2. Djelovanje obroka na koncentraciju Se u žumanjku, bjelanjku i cijelom jajetu

Dietary treatment Hranidbeni postupak	Se concentration, g/g, fresh basis[2] - Koncentracija Se g/g, svježa osnova[2]			
Se added, mg/kg Dodani Se	Source - Izvor	Yolk[3] - Žumanjak[3]	Albumen[3] - Bjelanjak[3]	Egg contents[3,4] Sadržaj u jajetu[3,4]
0		0.10	0.04	0.06
0.1	Na2SeO3	0.33	0.07	0.14
0.2	Na2SeO3	0.37	0.07	0.16
0.3	Na2SeO3	0.38	0.07	0.16
0.1	Se yeast - kvasac	0.32	0.08	0.15
0.2	Se yeast - kvasac	0.42	0.13	0.22
0.3	Se yeast - kvasac	0.48	0.15	0.25
SEM		0.012	0.013	0.011

1 From - Prema Paton et al. (2002)

2 Data presented are means for eggs from three groups of six hens, fresh basis - Predstavljeni podaci su prosjeci za jaja od tri skupine, šest kokoši, svježa osnova

3 Significant effect of Se supplementation (Basal vs. other diets) and of Se source (Na_2SeO_3 vs. Se yeast) (P < 0.05)- Značajno djelovanje dodatka Se (osnovnom i drugim obrocima) i izvor Se (Na_2SeO_3 naprama Se kvasca) (P < 0.05)

4 Values calculated from weights and Se concentrations of yolk and white - Vrijednosti dobivene iz mase i koncentracije žumanjka i bjeljanka.

In addition to the effects on embryonic Se levels, there were also significant increases in extra-embryonic Se concentration. There was a large increase in extra-embryonic Se concentration between days 15 and 20. However, it should be noted that the actual amount of Se in the extra-embryonic material is small at Day 20. The embryo would have absorbed a large amount of the extra-embryonic nutrients (other than Se) at this stage, thereby concentrating Se in the remaining extra-embryonic material.

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SAŽETAK

Ovaj rad jasno pokazuje da izvor i razina selena (Se) imaju velik utjecaj na količinu selena prenesenog u embrij u razvoju. Isto tako se čini da embrij apsorbira veće količine selena za vrijeme inkubacije od 10 do 15 dana nego u drugim razdobljima. Ovo može odražavati promjenljive potrebe za tim mineralom za vrijeme inkubacije, što može biti u svezi s fiziološkim i razvojnim procesima koji nastaju dok embrij raste. Poboljšanje prijenosa selena iz obroka/hrane kokoši upotrebom selenskog kvasca umjesto anorganskog natrijevog selenita je dobra strategija za poboljšanje hranidbenog stanja embrija kao i izvaljenog jajeta.

Ključne riječi: selen, izvor, embriji, Se kvasac, natrijev selenit