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Effect of Selenium-enriched Bean Sprout and Other Selenium Sources on Productivity and Selenium Concentration in Eggs of Laying Hens

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ABSTRACT : The objective of this study was to determine the effect of Se-enriched bean sprout, Se-enriched yeast and sodium selenite on productivity, egg quality and egg Se concentrations in laying hens. Using a Completely Randomized Design, 144 Rohman laying hens at 71 weeks of age were divided into four groups. Each group consisted of four replicates and each replicate contained nine hens. The dietary treatments were T1: control diet, T2: control diet plus 0.3 mg Se/kg from sodium selenite, T3: control diet plus 0.3 mg Se/kg from Se-enriched yeast, T4: control diet plus 0.3 mg Se/kg from Se-enriched bean sprout. The results showed that there was no significant difference ($p>0.05$) in feed intake, egg production and egg quality among treatments. Selenium supplementation from Se-enriched yeast and Se-enriched bean sprout markedly increased ($p<0.05$) egg Se concentration as compared to the control and sodium selenite groups. The results indicated that Se-enriched bean sprout could be used as an alternative Se source in diets of laying hens. (**Key Words** : Se-enriched Plant, Organic Selenium, Egg Selenium Content, Laying Hens)

INTRODUCTION

Selenium (Se) is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defense system, and immune function (Brown and Arthur, 2001). Interestingly, Se in selenoprotein or organic form has been found to be effective in reduction of cancer incidence in animal models, epidemiologic data and more recent studies in humans (Ganther, 1999; Diwadkar-Navsariwala and Diamond, 2004). Animal nutritionists have, therefore, paid more attention to supplementing Se to enhance productive performance and health of animals and to produce Se-enriched animal products to increase Se status of consumers. Concerning the latter, it was found that organic Se can readily accumulate into tissue protein in an unregulated manner (Thomson, 1998), resulting in higher Se concentration in animal products when compared to inorganic Se as reported in swine (Mahan and Parrett, 1996; Mahan et al., 1999; Olivera et al., 2005; Zhan et al., 2007), beef cattle (Lawler et al., 2004), dairy cows (Juniper et al., 2005; Muniz-Naveiro et al., 2005), broilers (Payne and Sounthern, 2005; Olivera et al., 2005; Yoon et al., 2007), Japanese quails (Sahin et al., 2008) and laying hens (Payne et al., 2005; Utterback et al., 2005; Skrivan et al., 2006; Pan et al., 2007; Chantiratikul et al., 2008). Currently, enrichment of eggs with Se is being produced in more than 25 countries worldwide, delivering approximately 30-35 μg Se or 50% of the recommended daily allowances (RDA) with a single egg (Fisinin et al., 2008). The Se-enriched eggs are produced using Se-enriched yeast as a major source of Se for laying hens at a level of 0.3-0.5 mgSe/kg in feed (Fisinin et al., 2008). However, the production process of Se-enriched yeast requires complex and high technology (Suhajda et al., 2000; Ouerdane and Mester, 2008). On the other hand, the production of Se-enriched plants is more practical (Sugihara et al., 2004; Tsuneyoshi et al., 2006). Selenium in the forms of selenate and selenite is readily absorbed by the plant and converted metabolically in the

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chloroplast to organic Se compounds (Terry et al., 2000), which are a component of protein in plant tissues (Leustek and Saito, 1999; Tinggi, 2003). Numerous studies revealed that Se-enriched plants could be successfully produced for human nutrition using edible plants such as broccoli sprouts (Finley et al., 2001), green onions (*Allium fistulosum*) (Kapolna and Fodor, 2006), garlic (Tsuneyoshi et al., 2006), and sprouts of several plants (Lintschinger et al., 2000; Sugihara et al., 2004). Additionally, these high-Se plants have anticarcinogenic activities (Finley et al., 2001; Yoshida et al., 2007). However, there is insufficient information on utilization of Se-enriched plants in terms of animal nutrition. Jiakui and Xiaolong (2004) produced Se-enriched malt and fed it to laying hens. They found that Se from sodium selenite and Se-enriched malt insignificantly deposited into eggs and productivity of the hens was not adversely affected. This result showed that Se-enriched plants could be used as a Se source in animal diets. Sprouts are normally utilized in foodstuffs of Asian people and the Se species in Se-enriched sprouts is mostly exhibited in organic form (Sugihara et al., 2004). Presently, the effect of Se-enriched sprout on laying hens has never been studied. Hence, this study was designed to compare the effect of Se-enriched bean sprout with other Se sources on productivity and Se concentration in eggs of laying hens.

MATERIALS AND METHODS

Se-enriched bean sprout was produced by cultivation of mung bean seeds (*Vigna radiata*) in opaque plastic containers (22×36×11 cm), containing cleaned sand after soaking the seeds in distilled water for 8 h. These cultivated seeds were applied with 400 ml distilled water containing 90 mg Se from sodium selenate/L. The container was fully covered by a black plastic bag. The bean sprouts were harvested after cultivation in the dark for 3 days, thoroughly washed with deionised water, dried at 50°C to constant weight and ground. Prior to preparation of dietary treatments, Se-enriched bean sprout and Se-enriched yeast (Alkosel®, Lallemand, Inc., Canada) were analysed for total Se by inductively coupled plasma-mass spectrometer (ICP-MS Model Elan-e, Perkin-Elmer SCIEX, USA) according to Joaquim et al. (1997).

The experiment was conducted in evaporative system housing with an internal temperature set at 24°C. Internal lights were on continuously. A total of 144 Rohman laying hens, 71 weeks old, were randomly allocated into 4 groups; each group contained 4 replicates with 9 hens per replicate and was placed in wire cages, three hens per cage. Feeders between the different cages were separated by plastic sheeting to avoid cross-contamination of dietary treatments. Water was freely available from nipple drinkers in the cages. All hens had been molted and returned to typical egg production levels before the start of the experiment. The control diet (Table 1) was formulated to meet the nutrient requirements of laying hens according to NRC (1994), without Se supplementation. Selenium from sodium selenite, Se-enriched yeast, and Se-enriched bean sprout was added to the control diet in a concentration of 0.3 mg Se/kg, which is commercially used in producing Se-enriched eggs (Fisinin et al., 2008). The hens received the control diet for a week prior to the beginning of the experiment and were fed dietary treatments *ad libitum* during 6 experimental weeks.

Feed consumption and egg production were recorded daily. The experimental diets were randomly collected at the end of each week, pooled by treatment, and analysed for chemical composition (AOAC, 1999) and Se content. Feed conversion rate was calculated as kilograms of feed consumed per kilogram of eggs. Eight eggs from each experimental group were sampled weekly (two eggs per replicate). Four collected eggs in each treatment were measured for egg weight, Haugh units, and eggshell thickness. Haugh units and eggshell thickness were measured using an albumen height gauge (TSS-QCD instrument, England) and a micrometer (395-541-30 BMD-25DM, Mitutoya, Japan), respectively.

Table 1. Feed ingredients and chemical composition of control diet[1]

Feed ingredients	% DM
Corn	59.00
Rice bran	4.25
Soybean meal (44% CP)	16.00
Fish meal	6.36
Soybean oil	2.78
Dicalcium phosphate	1.65
Oyster shell meal	8.44
DL-methionine	0.15
Salt	1.12
Vitamin-mineral premix[2]	0.25
Analyzed chemical composition	
Dry matter	83.84
Crude protein	16.41
Ether extract	1.39
Crude fiber	1.62
Ash	16.01
ME[3] (kcal/kg)	2,950

[1] Sodium selenite, Se-enriched yeast and Se-enriched bean sprout were mixed in corn and added to the diet to achieve the treatment levels.

[2] Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D_3, 11 mg vitamin E, 1.5 mg vitamin K_3, 1.5 mg thiamin, 4 mg riboflavin, 10 mg pantothenic acid, 0.4 folic acid, 4 mg pyridoxine, 22 mg niacin, 0.4 mg cobalamin, 0.1 mg biotin, 60 mg Fe, 70 mg Mn, 50 mg Zn, 8 mg Cu, 0.5 mg Co, 0.7 mg I.

[3] Calculated value.

Table 2. Selenium concentrations in selenium-enriched yeast, selenium-enriched bean sprout and dietary treatments

Items	Selenium (mg/kg)
Selenium-enriched yeast	2,117.08
Selenium-enriched bean sprout	223.45
Control diet	0.40
Control diet plus 0.3 mg Se/kg from sodium selenite	0.78
Control diet plus 0.3 mg Se/kg from selenium yeast	0.72
Control diet plus 0.3 mg Se/kg from selenium bean sprout	0.82

Whole egg Se concentration was determined in two eggs collected weekly in each treatment. The liquid eggs were weighed, homogenized well, dried at 65°C for 12 h and ground before determining Se concentration. Egg yolk and egg albumin of another two eggs were separated, dried at 65°C for 12 h and ground for Se analysis. Approximately 0.5 g of ground dietary treatments, whole egg, egg yolk and egg albumin were digested in a mixture of 1 ml HNO_3 and 9 ml deionized water until the solution was clear. Subsequently, the solution was diluted with deionized water to a final volume of 25 ml. Se was determined by inductively coupled plasma-mass spectrometer (ICP-MS Model Elan-e, Perkin-Elmer SCIEX, USA) according to Joaquim et al. (1997).

Statistical analysis

The data on feed intake, feed conversion rate, egg production, egg quality and Se concentrations in whole egg, egg yolk and egg albumin were analyzed by one-way ANOVA (SAS, 1996). The differences among means for each parameter were compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences were considered significant at $p<0.05$.

RESULTS

Se-enriched yeast and Se-enriched bean sprout contained 2,117.08 and 223.45 mg Se/kg, respectively. The actual concentrations of Se in the control diet and diets supplemented with 0.3 mg Se/kg from sodium selenite, Se-enriched yeast and Se-enriched bean sprout were 0.4, 0.78, 0.72 and 0.82 mg/kg, respectively (Table 2).

The results obviously demonstrated that feed intake, feed conversion rate/kg eggs, egg production and egg quality of laying hens were not negatively altered ($p<0.05$) by Se supplemental sources (Table 3).

Selenium concentrations in whole egg, egg yolk and egg albumin increased ($p<0.05$) with increasing dietary Se supplementation. Whole egg Se concentrations of laying hens fed Se supplemental diets from Se-enriched yeast and Se-enriched bean sprout were not significantly different ($p>0.05$), but higher ($p<0.05$) than those of hens fed the control diet and the Se supplemental diet from sodium selenite. Selenium from Se-enriched bean sprout dramatically accumulated ($p<0.05$) in egg yolk, however Se from Se-enriched yeast significantly increased ($p<0.05$) Se accumulation in egg albumin when compared to other sources of Se (Table 4).

Whole egg Se contents of hens fed Se supplemental diets from Se-enriched yeast and Se-enriched bean sprout were similar ($p>0.05$), but significantly higher ($p<0.05$) than those of hens fed the control diet and Se supplemental diet from sodium selenite (Table 4).

DISCUSSION

The results of feed intake, feed efficiency, egg production and egg quality in the current experiment are consistent with other studies comparing the effect of inorganic Se and Se-enriched yeast on laying hens (Payne et al., 2005; Utterback et al., 2005; Chantiratikul et al., 2008). Furthermore, Jiakui and Xiaolong (2004) found that the productivity of hens was not influenced by adding 0.51 mgSe/kg diet from sodium selenite or Se-malt. The dietary

Table 3. Effect of selenium sources on performance, egg production and egg quality of laying hens (n = 24)[1]

Items	C	SS	SY	SBS	SEM
Feed intake (g/d)	105.08	107.13	102.98	105.45	1.35
Feed conversion rate/kg eggs	1.86	1.95	1.92	1.90	0.03
Egg production (%)	75.60	73.08	70.43	75.40	1.76
Egg weight (g)	65.69	64.89	64.15	65.25	0.40
Haugh units (HU)	74.50	78.17	74.58	70.71	1.21
Eggshell thickness (mm)	0.33	0.33	0.34	0.33	0.004

[1] C = Control diet, SS = Control diet plus 0.3 mg Se/kg from sodium selenite, SY = Control diet plus 0.3 mgSe/kg from selenium yeast, SBS = Control diet plus 0.3 mg Se/kg from selenium bean sprout.

Table 4. Effect of selenium sources on selenium concentrations (mg/kg) in whole egg, egg yolk and egg albumin and selenium content in whole egg (μg/egg) of laying hens (n = 12)[1]

Items	C	SS	SY	SBS	SEM
Se concentration in whole egg	1.31^{c}	2.28^{b}	3.28^{a}	2.90^{a}	0.13
Se concentration in egg yolk	1.25^{c}	2.57^{b}	2.60^{b}	2.97^{a}	0.09
Se concentration in egg albumin	0.44^{d}	0.67^{c}	1.79^{a}	0.98^{b}	0.07
Se content in whole egg	17.60^{c}	29.47^{b}	42.61^{a}	39.18^{a}	1.78

[a, b, c] Means within same row with different superscripts differ (p<0.05).

[1] C = Control diet, SS = Control diet plus 0.3 mg Se/kg from sodium selenite, SY = Control diet plus 0.3 mg Se/kg from selenium yeast, SBS = Control diet plus 0.3 mg Se/kg from selenium bean sprout.

Se requirement and Se toxicity of laying hens have been recommended at 0.05 and 10 mg Se/kg diet, respectively (NRC, 1994). Ort and Latshaw (1978) revealed that a 9 mg Se/kg diet supplemented from sodium selenite resulted in a reduction of egg weight and egg production of laying hens. Although, the toxicity of organic Se in laying hens has not been directly reported, diets containing 20 mg Se/kg in selenomethionine form caused decreases in food consumption and growth of mallard duckling (Heinz et al., 1988). The present results indicated that supplementation of 0.3 mg Se/kg diet from Se-enriched bean sprout can be safely applied for laying hens without diminishing productivity.

Egg Se concentration is directly correlated to dietary Se supplementation and form of dietary Se (Golubkina and Papazyan, 2006). Consequently, egg Se concentration increased with increasing Se supplemental level, and organic Se was more effective for deposition into eggs than inorganic Se (Payne et al., 2005; Skrivan et al., 2006; Pan et al., 2007; Chantiratikul et al., 2008). The present results similarly reflected that Se from Se-enriched yeast and Se-enriched bean sprout had higher accumulation into the whole egg of laying hens than Se from sodium selenite (Table 4). Se-enriched yeast contains Se mainly in the organic form of selenomethionine (Whanger, 2002). Although, Se-enriched bean sprout used in the present study was not determined for Se speciation, previous studies (Finley et al., 2001; Sugihara et al., 2004) reported that the main Se species in the sprouts was Se-methyselenocysteine, which is a common metabolite from selenate or selenite in Se-enriched vegetables. The difference in major forms of organic Se in yeast and bean sprout was probably confirmed by Se accumulation in egg yolk and egg albumin (Table 4), indicating the different metabolic pathways of Se constituents in Se-enriched yeast and Se-enriched bean sprout. Absorbed selenomethionine can be incorporated non-specifically into proteins in place of methionine and also can be converted to selenocysteine that can be degraded to selenide. Similarly, methyselenol, which is a product of Se-methyselenocysteine catabolism, can be converted to selenide. The selenide finally enters the Se-protein synthetic metabolism (Comb, 2001). Additionally, selenomethionine can be produced by inorganic Se in animals, but its pathway is unknown (Whanger, 2002). The Se metabolism pathway obviously indicates that both organic and inorganic Se can be converted to selenoprotein. However, organic Se is more effective than inorganic Se in this respect (Thomson, 1998). Most published reports studied the effect of organic Se in yeast or in the form of selenomethionine and inorganic Se on Se concentration in eggs of laying hens (Paton et al., 2002; Payne et al., 2005; Pan et al., 2007; Chantiratikul et al., 2008). Only, Jaikui and Xiaolong (2004) found that, although Se in Se-malt was an organic form, Se deposition in whole egg of hens fed Se-malt or sodium selenite were not different (p>0.05). They concluded that Se in Se-malt was predominantly not in the form of selenomethionine. Additionally, Se in Se-enriched Chlorella (Skrivan et al., 2006) and spent compost of Se-enriched mushrooms (Lee et al., 2006) was actively bio-available to egg and muscle of beef steers, respectively. The aforementioned promising results demonstrated that various forms of Se can be supplemented in diets of animals. Therefore, Se-enriched plants should be further studied in terms of animal nutrition.

Generally, Se-enriched yeast is widely used in Se-enriched egg production, containing 30 to 35 μg Se/egg (Fisinin et al., 2008). Egg Se content of laying hens fed Se from yeast and bean sprout in the present study ranged from 39.18 to 42.61 μg Se/egg (Table 4), which is higher than that of commercial Se-egg. This was probably due to higher dietary Se concentration (0.72 to 0.82 mg Se/kg) when compared to that in diets (0.3 to 0.5 mg Se/kg) for Se-egg production (Fisinin et al., 2008). The results clearly indicate that Se-enriched bean sprout was comparable to Se-enriched yeast in producing Se-enriched egg.

CONCLUSION

Se-enriched bean sprout, Se-enriched yeast and sodium selenite did not alter (p>0.05) feed intake, egg production and egg quality of laying hens. Egg Se concentrations of laying hens fed Se supplemental diets from Se-enriched bean sprout and Se-enriched yeast were not different (p>0.05).

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