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Nutrition

Evaluation of the toxicity of selenium from hydroponically produced selenium-enriched kale sprout in laying hens

Anut Chantiratikul[a,*], Lalita Borisuth[a], Orawan Chinrasri[a], Nattanan Saenthaweesuk[a], Sumalee Chookhampaeng[b], Witphon Thosaikham[c], Noppong Sriart[d], Piyanete Chantiratikul[c]

[a] *Animal Feed Resources and Animal Nutrition Research Unit, Division of Animal Science, Faculty of Technology, Mahasarakham University, Maha Sarakham, Thailand*
[b] *Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham, Thailand*
[c] *Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Sciences, Mahasarakham University, Maha Sarakham, Thailand*
[d] *Mahasarakham University Farm, Mahasarakham University, Maha Sarakham, Thailand*

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ABSTRACT

Hydroponically produced Se-enriched kale sprouts (HPSeKS) are studied for their use as an alternative dietary Se supplement for poultry. The study experimented with different levels and sources of Se to determine toxicity and how the toxicity may affect productive performance, Se concentration in egg and tissues, and physiological responses of laying hens. One-hundred and twenty hens, 59 weeks of age, were divided into 5 groups. Each group consisted of 4 replicates and each replicate had 6 birds according to a $2 \times 2 + 1$ Augmented Factorial Experiment in a Completely Randomized Design. The experiment was conducted over a 4 week period, and 5 dietary treatments (T) were used: T1 basal diet, T2 and T3 basal diet plus 5 and 10 mg Se/kg from sodium selenite (SS), T4 and T5 basal diet plus 5 and 10 mg Se/kg from HPSeKS, respectively. The results make clear that Se from HPSeKS, at 5–10 mg/kg, did not affect ($P > 0.05$) feed intake and egg production; however, Se bioavailability decreased ($P < 0.05$). Egg, tissue and plasma Se concentrations, and GSH-Px activity in red blood cells increased ($P < 0.05$) compared to those in T1. Final body weight, feed intake, Se bioavailability, concentration of Se in breast muscle and plasma of hens fed Se from SS were lower ($P < 0.05$) than those of hens fed Se from HPSeKS. The findings demonstrate that dietary Se from HPSeKS at 5–10 mg/kg is not considered a toxic level for laying hens. The toxicity of Se from HPSeKS was less than the toxicity of Se from SS.

1. Introduction

Various available sources of organic selenium (Se) namely, Se-enriched yeast [1–4], selenomethionine [5–7], Se-enriched probiotics [8], heterotrophilically produced Se-enriched alga [9,10], and Se-enriched plants [11,12] have been investigated over time for use as a dietary supplement for poultry. Most of the studies conclude that the organic forms of Se have better bioavailability but are less toxic than inorganic forms. Recently developed hydroponically produced Se-enriched kale sprout (HPSeKS) using sodium selenite (SS) as a source of Se has become available. The sprouts of the kale predominantly consist of organic Se in selenomethionine (42%) and Se-methy-selenocysteine (35%) forms [13]. To determine the most acceptable source of Se, comparative studies involving Se from HPSeKS, Se-enriched yeast, and SS were conducted on quails and broilers. The comparative studies, which focused on increasing tissue Se concentration in poultry, revealed that Se from HPSeKS and Se-enriched yeast increased tissue Se concentration at nearly the same level; whereas, the Se from SS increased concentration at a lower level [14,15]. This finding clearly indicates that Se from HPSeKS could effectively be used in poultry diets as a source of Se. With respect to toxicity and tolerance, Se is generally considered the most toxic of essential trace elements [16], and as such, a dietary restriction of 0.1 mgSe/kg is recommended for laying hens [17]. The maximum level for poultry is limited to 0.2–0.5 mg/kg [18,19]. The tolerance and toxic levels of dietary Se in poultry are suggested to be 5 and 10 mg/kg, respectively [17]. Scientific literature reveals that

* Corresponding author at: Division of Animal Science, Faculty of Technology, Mahasarakham University, Kantharawichai, Maha Sarakham, 44150, Thailand.
E-mail addresses: c_anut@hotmail.com, anut.c@msu.ac.th (A. Chantiratikul).

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

Ingredients	g/kg
Corn	580.0
Extruded soybean	97.0
Soybean meal (44%CP)	167.5
Soybean oil	37.5
Dicalcium phosphate	15.0
Oyster shell meal	85.0
DL-methionine	3.0
L-lysine	1.0
Salt	11.5
Vitamin-mineral premix[b]	2.5
Analyzed chemical composition	
Dry matter	914.2
Crude protein	167.1
Ether extract	71.4
Crude fiber	22.8
Ash	140.2
ME[c] (MJ/kg)	12.34

[a] SS and HPSeKS (355 mgSe/kg) were mixed in corn and added to the diet to achieve the treatment levels.
[b] Premix provided (per kg of diet): 10,000 IU vitamin A; 2,000 IU vitamin D3; 11 mg vitamin E; 1.5 mg vitamin K3; 1.5 mg thiamin; 4 mg riboflavin; 10 mg pantothenic acid; 0.4 folic acid; 4 mg pyridoxine; 22 mg niacin; 0.4 mg colabamin; 0.1 mg biotin; 60 mg Fe; 70 mg Mn; 50 mg Zn; 8 mg Cu; 0.5 mg Co; 0.7 mg I.
[c] Calculated value.

a dietary of 5 mgSe/kg is borderline for reducing hatchability. Supplementation of 7–9 mgSe/kg was found to impair appetite, growth and egg production [20]. High mortality rates of poultry occurred with Se addition at 30–40 mg/kg. The Se from Se-enriched yeast was not as toxic as the Se from selenomethionine and SS [21]. Given there is no available information on the toxicity level of Se from HPSeKS for poultry, the purpose of this trial was to evaluate the effects of toxicity of Se from HPSeKS on productive performance, Se content in egg and tissues, and physiological responses of laying hens. Se from SS was chosen to be the comparative source.

2. Materials and methods

2.1. The production of Se-enriched kale sprouts under hydroponic system

Cleaned kale seeds (*Brassica oleracea* var. *alboglabra* L.) were soaked in tap water for 15 h, cultivated into wet sponges and placed in plastic pots (35 × 40 × 30 cm). The pots were fully covered for 3 days until the seeds germinated. For 4 days, the germinated seeds were subjected to light from a fluorescent lamp (36 W) from 06.00 h to 18.00 h and watered daily with tap water. The germinated seeds were then planted in an adapted Hoagland's solution containing 30 mgSe from SS/L. A 15 day cultivation period under the hydroponic system was then set in motion. Following the 15 day period, HPSeKS were harvested, washed thoroughly with deionized water and dried at 60 °C in a hot air oven, ground, and stored at 4 °C [14].

2.2. Birds and experimental procedures

The experimental procedures were approved by the Animal Ethics Committee of Mahasarakham University (Approval No. 001/2558).

One hundred and twenty Betagro laying hens were housed in a large enclosure with an evaporative cooling system. The 59-week-old hens were randomly divided into 5 groups, according to a 2 × 2 + 1 Augmented Factorial Experiment in a Completely Randomized Design. Each group consisted of 4 replicates with 6 hens per replicate. The basal diet (Table 1) was formulated to meet nutrient requirements, recommended by NRC [17], without Se supplementation. The dietary treatments (T) were T1: basal diet, T2 and T3: basal diet supplemented 5.0 and 10.0 mgSe/kg from SS (Na_2SeO_3), T4 and T5: basal diet supplemented 5.0 and 10.0 mgSe/kg from HPSeKS, respectively. The hens were fed basal diets or Se supplemented diets and water *ad libitum* throughout 4 experimental weeks.

2.3. Data and sample collections

The dietary treatments were randomly collected, dried and ground. Initial body weights were measured at the beginning, and final body weights were measured the end of the trial for estimation of body weight changes. Feed intake and egg production in each replicate were examined daily.

Four eggs per replicate of each treatment were randomly collected on day 1–3 and at the end of week 1–4 and stored at 4 °C until processed. Eight sampled eggs from each collection period were analyzed for egg weight, Haugh units, yolk color and eggshell thickness. Haugh units were estimated by measuring albumen height with an albumen height gauge (albumen height gauge TSS-QCD instruments, England) and calculated according to Haugh [22]. Eggshell thickness and yolk color were measured with a micrometer (395-541-30 BMD-25DM, Mitutoya, Japan) and a yolk color fan (Roche yolk color fan 1993-HMB 50515, Switzerland), respectively. Another eight eggs were prepared for Se determination. The first four of these eggs were broken and mixed. Egg albumen and yolk of the remaining four eggs were separated and homogenized. Then, samples of the whole egg, egg albumen and yolk were dried at 65 °C for 12 h, ground and equally pooled by replicate in each treatment and stored.

The hen's excreta output from each replicate was collected in a pan located underneath the cages. The collections were made on two consecutive days in week 4. After collection, the excreta was weighed, kept at 60 °C in a drying oven for 24 h until dry, weighed, ground and stored.

On day 28, blood samples of two hens in each replicate were drawn by puncturing the wing vein. The samples were then placed into sterile test tubes with EDTA. Blood samples were centrifuged at 3000 × *g* for 10 min. The plasma was harvested and stored at −20 °C. Afterwards, red blood cells (Rbc) were washed three times with 0.9% saline solution. Distilled water was added to the Rbc in a ratio of 4:1 and frozen for 24 h to hemolyse the Rbc [23].

At the end of the trial, 2 hens per replicate of each treatment were randomly selected and slaughtered by cervical dislocation. The liver, kidney, heart and breast muscle were harvested, dried, ground, equally pooled by replicate and stored.

2.4. Chemical analysis and calculation

The dietary treatments were analyzed for chemical composition [24]. The dried samples of HPSeKS, diet, whole egg, egg albumen, egg yolk, excreta, plasma, liver, kidney, heart and breast muscle were weighed accurately and placed into a vessel. One point five mL of 70% w/w nitric acid and the same volume of deionized water were then added to each sample to carry out the acid digestion procedure. The samples were digested at 100 °C in the digestion block until the solution dried and cooled. In the second step, after the vessel cooled, 5 mL of HCl was added to reduce Se (VI)–Se (IV). Next, the samples were heated at 100 °C for 10 min and cooled. Finally, deionized water was poured into the volumetric flask to make up the reduced volume of the digest. The total amount of Se in the samples was determined using a Hydride Generation Atomic Absorption Spectrometer (HG-AAS model VGA-77, Agilent Technologies, Inc., USA) at maximum wavelength of 196.0 nm, quartz cell temperature 850 °C, carrier solution HCl 4 M and $NaBH_4$ 0.2% m/v. The digestion method was accurately validated with a recovery test by spiking 50 μL of a Se standard solution (200 mgSe/L)

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