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Selenium-Fertilized Tritordeum (× Tritordeum Ascherson et Graebner) as Dietary Selenium Supplement in Laying Hens: Effects on Egg Quality

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Abstract The aim of the present study was to investigate the effect of adding selenium (Se) in cereal production by fertilization on Se concentration in laying hen eggs. Tritordeum (×Tritordeum Ascherson et Graebner), a new cereal from the cross between durum wheat and a wild barley species having accreditation as natural crop species, was produced using selenate as Se-fertilizer. Hy-Line Brown laying hens were randomly allocated to two dietary treatments and fed for 10 weeks. Hens were fed two corn-soybean meal-based diets comprising a control basal diet including Tritordeum (100 g/kg diet) cv. Aucan grown without Se fertilization (containing background Se only from premix supplying 1,0 times birds' requirements) and a test-diet containing Se-enriched Tritordeum at the same level of the control diet. No difference was observed among dietary treatments on feed consumption and efficiency, egg mass, and laying rate, whereas egg yolk Se and vitamin E contents as well as liver and plasma Se levels were significantly influenced by dietary Se-enriched Tritordeum. Based on our findings, Se-enriched Tritordeum improved egg quality without affecting hens' productive performance. Thus, Se-fertilized Tritordeum may represent a valuable natural source of Se compared to conventional dietary supplements.

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Introduction

Selenium (Se) is a dietary essential nutrient for animals as well as for humans [1–3]. It is vital for the antioxidant enzyme glutathione peroxidase function, which protects the cells by destroying free radicals [4–6]. The Se requirement for laying hens ranges from 0.05 to 0.08 ppm depending on daily feed consumption [1], and this Se requirement can be met by a common corn-soybean meal diet without additional supplementation. However, Se content of feed grains widely varies from region to region [7, 8], and thus, it is a common practice in the poultry industry to supplement laying hen diets. Recommended Se levels in cereals are in the range of 0.2–0.3 ppm Se on dry matter for livestock species [7]; however, in many countries, the plants availability of Se in soil is low, resulting below the standard concentrations. Thus, to guarantee enough nutritional levels, Se-enriched supplements may be utilized, and an alternative could be the application of Se fertilizers to crops as it has been practiced satisfactory in Europe on wheat [9, 10].

Tritordeum (×Tritordeum Ascherson et Graebner) is a new and fertile amphiploid derived from the crosses between *Hordeum chilense* Roem. et Schultz, as the maternal parent, and either tetraploid or hexaploid wheat [11]. Tritordeum exhibits agronomic, morphological, chemical, physicochemical, and rheological characteristics similar or better to wheat, and it is well adapted to Mediterranean environments [12–14]. These properties, together with the enormous genetic variability potentially available for breeding this new crop, make tritordeum a promising cereal for agriculture and food processing [15]. Since in many countries Se is scarcely present in soils, its concentration in animal and human diet should be

enriched in a capable way [7]. In this way, the role of Se-fertilized tritordeum as dietary Se supplement in poultry has never been investigated. Therefore, based on previous considerations, the present study aimed to investigate the biological effects of Se in a diet containing Se-fertilized tritordeum for laying hens on their productive performance, egg quality, and Se concentrations in egg, plasma, and liver as indicators.

Materials and Methods

This study was conducted in the experimental poultry facility located at the University of Bari "Aldo Moro", Italy, observing the animal welfare Legislative Decree 116/92, Council Directive 98/58/EC, received in Italy by Legislative Decree 146/2001, and Council Directive 2007/43/CE, received in Italy by the governmental Decree 181/2010 and Legislative Decree 267/2003.

Tritordeum Production for the Diets

The trial was conducted at Putignano (Bari) in Apulia region, Southern Italy (longitude 17° 7′ E, latitude 40° 51′ N, elevation 310 m above sea level) on a sandy clay textured soil (60 % sand, 31 % clay) characterized as sub-alkaline (pH 7.2), medium in total nitrogen (1.12 ‰), high in available phosphorus (94 ppm), and exchangeable potassium (335 ppm).

Tritordeum (× Tritordeum Ascherson et Graebner) cv. Aucan was sown on 15 November 2013 at a seeding rate of 180 kg ha^{-1} and a row spacing of 15 cm on a plot area of 3000 m^2. Selenium was applied (100 g Se ha^{-1}) on a plot area of 1500 m^2, as a solution of Na_2SO_4 at plant early-boot stage and Growth Stage no. 41 [16] in April. During the cropping cycle (2013–2014), herbicides were applied as in standard farming practices in Italy and nitrogen fertilization with 100 kg ha^{-1} of N as urea (4 March) was performed. The crop was harvested at maturity, and the Tritordeum was used for the feeding trial.

Dietary Treatments

A total of 120 Hy-Line Brown laying hens, 25 weeks of age with an initial average body weight of 1610 ± 11.5 g, were free-range reared. The hens were divided into two groups of 60 hens each and were housed in different indoor pens (0.15 m^2/bird) equipped with feeders and drinkers, with free access to open air runs (3 m^2/bird).

Laying hens were fed two corn-soybean meal-based diets comprising a control basal diet including Tritordeum (× Tritordeum Ascherson et Graebner) cv. Aucan (100 g/kg diet) grown without Se fertilization and a test diet containing Se-enriched Tritordeum at the same level of the control diet. The control diet containing background Se only in the premix (Table 1) supplied 1,0 times birds' daily requirements. The basal diet contained 17 % crude protein (CP) and 2836 kcal of metabolizable energy (ME)/kg of diet, designed to meet or exceed the nutrient requirements for laying hens [1]. The Se concentration in unfertilized Tritordeum was 0.105 mg/kg of dry matter (DM), whereas it was 0.580 mg/kg of DM in Se-enriched Tritordeum, respectively. The calculated value of added plus natural Se in the experimental diet was 0.258 mg/kg of DM. Each diet was replicated four times, with each replicate comprising one pen of 15 hens. Feed and water were provided ad libitum throughout the entire trial. Hens' mortality rate was recorded if it occurred.

Table 1 Ingredients and chemical analysis of basal diet fed to laying hens

Ingredients, g/kg as-fed basis	Basal diet
Corn	521.0
Soybean meal (48 % CP)	153.0
Tritordeum	100.0
Calcium carbonate	90.0
Alfalfa meal (17 % CP)	40.0
Corn gluten meal (60 % CP)	40.0
Sunflower oil	25.0
Dicalcium phosphate	17.0
Vitamin-mineral premix[a]	2.5
Sodium chloride	2.5
Sodium bicarbonate	2.5
DL-Met	2.0
L-Lys HCl	2.0
Yeast	1.5
Thr	1.0
Chemical analysis, %	
Dry matter	87.54
Crude protein	16.85
Crude fiber	3.22
Crude fat	5.74
Ash	13.21
Calculated analysis	
ME, MJ/kg	11.87
Lys, %	0.86
Ca, %	4.05
Met + Cys, %	0.74
Available P, %	0.36

[a] Provided per kg of product: 2500.000 IU vitamin A; 300,000 IU vitamin D_3; 7500 mcg 25-hydroxycholecalciferol; 6000 mg vitamin E; 500 mg vitamin K_3; 4000 mg vitamin PP; 300 mg vitamin B_1; 1000 mg vitamin B_2; 2000 mg D-pantothenic acid; 400 mg vitamin B_6; 3 mg vitamin B_{12}; 150 mg folic acid; 20 mg D-biotin; 10,000 mg Fe; 1000 mg Cu; 30,000 mg Mn; 40 mg Co; 15,000 mg Zn; 200 mg I; 20 mg Se (as sodium selenite)

Sample Collection and Procedures

Diet samples were ground in a hammer mill with a 1-mm screen and analyzed in triplicate for dry matter (DM, 945.15), ash (967.05), crude protein (CP, Kjeldahl N × 6.25, 990.03), crude fiber (978.10), and ether extract (945.16) according to AOAC [17] methods. The NDF (using heat-resistant α-amylase without sodium sulphite), ADF, and ADL (lignin) were analyzed according to Mertens [18], AOAC [17] (973.187), and Van Soest et al. [19], respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York).

Eggs were daily collected and weighted, and egg production was calculated on a hen-day basis. Egg mass was calculated as egg production × egg weight. Eggs with any adhering manure were classed as dirty, and the percentage was calculated. Feed intake was recorded weekly by replicate. Feed conversion ratio (FCR) was calculated as g of feed/g of egg mass. Eggs were analyzed for their interior and exterior quality as reported by Laudadio and Tufarelli [20]. Eggs were examined for shell quality by specific gravity. Shell thickness (with shell membrane) of the eggs (20 % of the daily egg produced) was measured by micrometer. Shell thickness was a mean value of measurements at three locations on the eggs (air cell, equator, and sharp end). Breaking strength of uncracked eggs was determined with a testing machine (model 1140, Instron Ltd., Bucks, UK). Egg components (albumen, yolk, and shell) were measured by weekly breakouts on two eggs per replicate pen and expressed as percentage of egg weight. Haugh unit was calculates as: Haugh units = $100 \times \log (H + 7.57–1.7\ W^{0.37}$, where H is the height of the albumen and W is the weight of the egg) according the formula proposed by Card and Nesheim [21]. Egg yolk color was scored using the 15-point scale (color scale from 15, dark orange to 1, light pale) of the DSM yolk color fan (DSM Nutritional Products Ltd., Basel, Switzerland).

Egg Yolk, Liver, and Blood Se Analysis

During the whole feeding trial duration (from day 0 and then weekly), three eggs per replicate were randomly selected for egg yolk Se analysis. These eggs were marked with their treatment and replicate pen number and then stored in a cooler. The eggs were cracked and the shells were discarded. The liquid egg yolks were mixed and homogenized with a malt blender and stored frozen in 12-ml plastic cups until Se analysis. Total Se content in feed, egg yolk, plasma, and liver were assessed (Agilent 4100 MS-AES, Agilent, Santa Clara, CA, USA).

Egg yolk vitamin E content was extracted using the method described by Abdollahi et al. [22], and HPLC determination was performed according to the conditions described by Mohiti-Asli et al. [23]. All analytical procedures were conducted following the UNI CEI EN ISO/IEC 17025:2005 standards.

Individual blood samples (1.5 ml) were weekly collected during the whole feeding period. Blood was collected from the brachial wing vein using sterilized syringes and needles. After 1 h standing at room temperature, serum was isolated by centrifugation at 1150×g for 10 min at 20 °C. Serum samples were stored at −80 °C until further analysis. At the end of the feeding trial, liver of three hens in each replicate was sampled after slaughtering and frozen for further analyses.

Statistical Analysis

One-way ANOVA option of the GLM of SAS/STAT software [24] was used to analyze data as a completely randomized design with the dietary treatment as main effect. The statistical model used was $Y_{ijk} = \mu + P_i + R_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = response variables from each individual replication or pen; μ = the overall mean; P_i = the effect of dietary treatment; R_{ij} = the inter-experimental unit (replications) error term; and ε_{ijk} = the intra-experimental unit error term. When there was a significant F-value, means were compared by the Student-Newman-Keul's method. Unless stated otherwise, significance level was set at $P < 0.05$.

Results and Discussion

The dietary effects of Se-fertilized Tritordeum on laying hens feed intake and efficiency and egg productive and qualitative parameters are reported in Table 2. Diet-containing Se-fertilized Tritordeum did not significantly influence feed intake; moreover, the feed efficiency was also similar between the two dietary groups. Regarding the other laying hens' performance parameters investigated, comprising egg production rate, egg weight, and mass were not significantly affected by the new cereal enriched with Se in the hens' diet. These

Table 2 Effect of diets on laying hens' feed intake and efficiency, egg laying rate, and weight

Item	Diet[a]			
	Control	Tritordeum-Se	SEM	P value
Feed intake, g/day	117.6	118.4	0.23	0.298
Egg weight, g	61.4	62.1	0.20	0.087
Egg mass, g/day	53.7	54.7	0.20	0.076
FCR[b], g feed/g egg mass	2.04	2.05	0.07	0.182
Laying rate, %	87.5	88.1	0.15	0.303

[a] Control = basal diet containing unfertilized Tritordeum; Tritordeum-Se = basal diet containing Se-fertilized Tritordeum

[b] FCR = feed conversion ratio

findings agree with previous reports on dietary Se supplementation using enriched plants in laying hens as well as in broiler chickens [7, 8]. Jiakui and Xiaolong [25] found that the metabolic path of Se from Se-enriched barley (*Hordeum vulgare*) was comparable to that of Se from sodium selenite in laying hens, indicating that selenomethionine was not the predominant Se form in the studied cereal. Conversely, Chinrasri et al. [26] reported that Se from Se-enriched kale sprout (*Brassica oleracea var. alboglabra* L.) did not influence performance characteristics in quails, and Se from Se-enriched kale sprout offered no advantage over Se from sodium selenite on tissue Se concentration. Furthermore, Chinrasri et al. [27] found that Se-enriched bean sprout (*Vigna radiate*) was comparable to Se-enriched yeast in egg Se concentration of laying hens. Similarly to our results, recent studies conducted on laying hens and broiler chickens have shown that dietary Se supply did not influence growth performance parameters such as live body weight and gain, as well as feed intake [28, 29].

Dietary treatment did not negatively influence any parameters related to egg or shell quality (Table 3). The overall values obtained in the present study are quite acceptable for optimal egg quality for this age of hens (25 to 35 weeks) in the medium production phase. The percentage of different egg components (yolk, albumen, and shell) during the trial did not differ among dietary treatments. Moreover, Haugh units were also similar. The shell thickness and strength had similar mean values among dietary treatments for each parameter, indicating a similar relative density for produced eggs. The average values of the obtained egg quality parameters conformed to the data reported for standard commercial egg production guides and other available literature [23,29,30). The egg yolk color score was similar when Se-fertilized Tritordeum as Se-supplement was included into the laying hens diet compared with control group.

In the present study, Adding Se fertilized tritordeum to diet resulted in significant increase of Se concentrations in yolk in the experimental group fed compared with the control laying hens (Table 3), resulting in line with the USDA recommendations [31]. In fact, selenium has received as much attention as one of most important egg yolk components because of its continuing interest for human and animal nutrition [32, 33].

Many previous trials have evaluated the opportunity of enriching the Se concentration of various dietary items, including meat and eggs. In fact, eggs have been shown to be an effective vehicle for supplementing Se in the diet [23, 28, 30]. The egg Se level is efficiently modified when laying hens are fed diets with different sources forms of Se. Selenium-enriched eggs have been shown to be a valuable source of Se for humans as well as for livestock animals [33]. Moreover, eggs are a traditional and not expensive food in many countries; as a result, Se-fortified eggs should be positively agreeable.

Feeding of Se-fertilized Tritordeum in diet significantly increase egg yolk total vitamin E concentration (Table 3). The amount of vitamin E deposited in egg depends upon the dietary level and birds age [34]. The observed increase in vitamin E concentrations in the egg yolk from laying hens fed Se-supplemented diet is in agreement with the findings of previous trial using other organic different Se sources [23,

Table 3 Effects of dietary treatments on egg quality parameters and Se concentration in egg yolk and hens' liver and plasma

Item	Diet[a]			
	Control	Tritordeum-Se	SEM	*P* value
Egg components, %				
Yolk	23.7	24.0	0.09	0.404
Albumen	63.3	63.8	0.13	0.363
Shell	13.0	12.2	0.06	0.101
Haugh unit	88.20	88.39	0.17	0.337
Shell thickness (mm × 10^{-2})	0.30	0.33	0.01	0.515
Shell strength (kg/cm^2)	1.59	1.61	0.03	0.222
Broken + shell-less eggs, %	0.18	0.14	0.02	0.688
Dirty eggs, %	0.35	0.31	<0.01	0.677
Yolk color score	11.88	12.01	0.15	0.466
Egg yolk				
Selenium, ppm wet weight	0.129	0.227	0.19	0.018
Vitamin E, ppm wet weight	0.788	0.954	0.23	0.006
Selenium in liver, ppm wet weight	0.191	0.249	0.07	0.011
Selenium in plasma, mg/l	0.250	0.318	0.02	0.019

[a] Control = basal diet containing unfertilized Tritordeum; Tritordeum-Se = basal diet containing Se-fertilized Tritordeum

30]. Supplementing Se-fertilized Tritordeum in laying hen diet led to a significant improvement of Se levels in liver as well as in plasma compared to the control diet (Table 3). Thus, feeding the Tritordeum as dietary Se source resulted in more Se deposition. Earlier reports have indicated that the increase in Se concentrations in egg and tissues depends on the Se source, but the results of these trials were somewhat variable [8]. In agreement to our findings, Hossain et al. [35] showed that Se from Se-enriched Japanese radish sprout gave variable outcomes on yolk and tissue Se levels in laying hens. Combs and Combs [36] reported that organic Se sources, such as selenium yeast, are actively absorbed and can be directly incorporated into protein, whereas, inorganic Se sources, such as sodium selenite, are passively absorbed by the body. Therefore, according to the present study, it assessed the effectiveness of Se-fertilized Tritordeum as Se supplement for laying hens.

Conclusion

Based on our findings, the present research demonstrated the possibility to use Se-fertilized Tritordeum as a new natural dietary Se source than conventional inorganic sodium selenite supplement due to its good bioavailability and capacity to increase the Se level in egg yolk, plasma, and liver without affecting hens' productive performance. In addition, the higher levels of this trace element could defend lipids from oxidation enhancing the quality of the animal-derived products.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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