Influence of dietary vitamin C and selenium, alone and in combination, on the performance of laying hens and quality of eggs

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ABSTRACT: Two hundred and forty laying hens were fed diets supplemented with vitamin C and selenium (Se). Vitamin C was added at 0 or 200 mg/kg, and Se was added as sodium selenite or selenized yeast at 0.3 mg/kg. The feed intake and egg production were measured, and egg quality parameters were determined. Supplementation of the basal diet with Se significantly increased the laying performance; however, vitamin C significantly decreased feed intake and egg production. Vitamin C increased vitamin E concentration in the yolk. Both selenite and Se-enriched yeast increased the vitamin E concentration in the yolk and the Se concentration in the yolk and albumen. The oxidative stability of yolk lipids was improved in hens fed diets supplemented with sodium selenite, but not in those fed diets supplemented with Se-yeast. After 28 days of storage, however, the beneficial effect of selenite on lipid stability ceased. The supplementation of the basal diet with vitamin C significantly worsened the oxidative stability of yolk lipids, indicating that vitamin C acted as a pro-oxidant. Thus, vitamin C increased the laying performance and influenced some traits of egg quality. The combined supplementation of vitamin C and Se did not prove to be successful.

Keywords: ascorbic acid; sodium selenite; selenized yeast; layers; vitamins; egg production

Vitamin C (L-ascorbic acid) is required for a range of metabolic reactions in animals, in particular for the scavenging of reactive oxygen species as well as the synthesis of collagen, adrenalin, and bile acids (Mayes, 2002). Fowl possess the ability to synthesize vitamin C; however, various stressful environmental, nutritional, and pathological conditions may increase the metabolic need for vitamin C beyond the synthetic ability. There are numerous reports on the use of vitamin C in poultry. Dietary supplementation with vitamin C is beneficial for birds subjected to poor nutrition, invaded with pathogens, and exposed to either elevated or reduced environmental temperature (Pardue and Thaxton, 1986). Among chickens fed nutritionally adequate diets, vitamin C does not consistently stimulate growth (McKee and Harrison, 1995). In the first weeks after hatching, however, the biosynthesis of ascorbic acid is not yet fully developed, and feed supplementation of 100–300 mg vitamin C/kg may improve the growth and functional capacity of the immune system (Kolb and Seehawer, 2001).

Reports on the effect of vitamin C supplementation on the performance of laying hens is inconsistent. Pardue and Thaxton (1986) concluded that supplementing the diets of hens was of greatest benefit when hens were exposed to environmental or nutritional stress whereas supplementing diets of laying hens with vitamin C when environmental conditions were mild was of little value. Keshavarz (1996) observed that a diet supplemented with vitamin C at 250 mg/kg had no effect on eggshell quality, but egg weight was increased. Salvador et al. (2009) and Wang et al. (2011a) reported no beneficial effects of vitamin C on egg weight and egg production in laying hens. In broiler breeders, however, 50 and 100 mg/kg vitamin C feed supplements significantly increased hen-day

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egg production and hatching of fertile eggs. Much of the improvement in the hatching of fertile eggs was due to a decrease in early embryonic mortality (Peebles and Brake, 1985).

Selenium (Se) is an essential trace element that is required for the activity of selenoproteins involved in antioxidant defence, thyroid hormone deiodination, and other vital physiological functions (Surai, 2006). Several authors have studied the effect of a combination of Se and vitamin E on the antioxidative system in broiler chickens (Surai, 2000; Skřivan et al., 2008; Kim et al., 2010) and laying hens (Skřivan et al., 2010). Skřivan et al. (2012) investigated the combined effect of dietary vitamin C and Se on the composition and oxidative stability of the meat of broiler chickens. Both vitamin C and Se improved oxidative stability of thigh meat stored at 4°C for 5 days. The lowest concentration of thiobarbituric acid-reactive substances (TBARS, i.e. degradation products of lipids) was observed in the meat of chickens fed vitamin C at 280 mg/kg and sodium selenite at 0.3 mg Se/kg feed. Information on the combined effect of supplemental vitamin C and Se on the performance of laying hens and the quality of eggs is not available in the literature. The objective of the present study was to evaluate the effect of supplementing vitamin C and Se on the performance and quality of eggs from laying hens fed a wheat-maize-soybean diet. Se was supplied as sodium selenite or selenized yeast.

MATERIAL AND METHODS

Diets and husbandry

Two hundred and forty ISA Brown laying hens, 20 weeks of age, were randomly allocated to 6 groups and housed in the same air-conditioned facility at 10 hens per cage. The room temperature was maintained at 20–22°C, and the light cycle consisted of 15 h of light and 9 h of darkness. The cages were equipped with a nest box, perch, dust bath, and equipment for sharpening claws. The experimental design involved a 3 × 2 factorial arrangement of treatments with two levels of supplemental vitamin C and three sources of Se: a basal wheat-maize-soybean diet containing 0.09 mg Se/kg (Table 1), a basal diet with sodium selenite added at 0.3 mg Se/kg, and a basal diet with Se-enriched yeast (Sel-Plex) (Alltech, Lexington, USA) added at 0.3 mg Se/kg. Vitamin C (ROVIMIX® C-EC) (DSM Nutritional Products, Heanor, UK) was added to the diets at 0 and 200 mg/kg. The diets were then pelleted and stored in a dark room at 18°C for 12 weeks and then new diets were prepared. The experiment duration was 24 weeks.

Table 1. Composition of the basal diet[a]

Ingredients (g/kg)		Analyzed composition	
Wheat	32.00	dry matter (g/kg)	899
Maize	31.90	crude protein (g/kg)	171
Soybean meal, extracted	18.50	ether extract (g/kg)	44.9
Rapeseed oil	2.50	crude fibre (g/kg)	33.3
Alfalfa meal	2.00	ash (g/kg)	111
Wheat bran	1.50	calcium (g/kg)	37.1
Fish meal	1.50	phosphorus (g/kg)	5.8
Limestone (0.1–1.0 mm)	8.20	iron (mg/kg)	130
Calcium hydrogenphosphate	0.96	selenium (mg/kg)	0.09
Sodium chloride	0.20	vitamin A (mg/kg)	2.7
DL-Methionine	0.13	vitamin E (mg/kg)	42.7
L-Lysine HCL	0.11	vitamin C (mg/kg)	3.5
Vitamin-mineral premix[b]	0.50	ME (MJ/kg), calculated	11.4

[a]experimental diets were supplemented with vitamin C at 200 mg/kg and with Se at 0.3 mg/kg

[b]premix provided per kg of diet: vitamin A 3 mg, vitamin D_3 0.075 µg, vitamin E 30 mg, vitamin K_3 2 mg, vitamin B_1 2 mg, vitamin B_2 5 mg, vitamin B_6 3 mg, vitamin B_{12} 0.015 mg, niacin 25 mg, Ca-pantothenate 8 mg, biotin 0.075 mg, folic acid 0.5 mg, choline chloride 25 mg, betaine 50 mg, Fe 40 mg, Mn 70 mg, Zn 50 mg, I 1 mg, Co 0.3 mg, butylated hydroxytoluene 50 mg

Data collection, sampling, and measurements

Feed intake (per cage) was recorded weekly. Eggs were collected daily. Each week, all eggs were weighed in three consecutive days. Once every 4 weeks, all eggs were collected for analyses of physical parameters of egg quality; a total of 1082 eggs were analyzed during the experimental period. Weight, shell parameters, albumen height, and Haugh units were measured for each egg on the day of collection. Shell breaking strength was determined on the vertical axis using an Instron 3360 testing system (Instron, Canton, USA). Eggs were broken, and the albumens and yolks were separated and weighed. Eggshell thickness was measured with a micrometer as the average of both ends and at the middle, including the shell membranes. The shells with the membranes were washed, dried at 105°C, and weighed. The eggshell index was calculated after Ahmed et al. (2005) as follows:

$$SI = (SW/S) \times 100$$

where:
SI = eggshell index (g/100 cm^2)
SW = shell weight (g)
S = shell surface (cm^2) calculated as S = 4.68 × egg weight $(EW)^{2/3}$ (g)

Albumen height was measured using a tripod micrometer (Keener et al., 2006). Haugh units were calculated as described by Haugh (1937). The colour parameters of the yolk (L^*, a^*, b^*) were measured using a CR-300 colorimeter (Konica Minolta, Osaka, Japan).

Analyses

The feed was analyzed as described previously (Marounek et al., 2008). To determine the Se content, samples of the feed, yolk, and albumen (n = 36) were digested in a mixture of HNO_3 and H_2O_2 in Teflon high-pressure vessels in a MDS-2000 microwave oven (LabX, Midland, Canada). After mineralization, the Se was quantified by fluorometric method (AOAC, 2005; method 996.16), employing the Millennium Excalibur system (PS Analytical, Orpington, UK). The procedure was validated by an analysis of certified reference material RM 8415 Whole Egg Powder (National Institute of Standards and Technology, Gaithersburg, USA). The Fe concentration in the mineralized feed and yolk (n = 36) was determined by atomic absorption spectrometry using Solaar M6 instrument (TJA Solutions, Cambridge, UK). The vitamin E (α-tocopherol) and vitamin A (retinol) contents of the feed and egg yolks (n = 36) were determined according to the European standards EN 12822 (2000) and EN 12823-1 (2000) by high-performance liquid chromatography (VP series; Shimadzu, Kyoto, Japan) equipped with a diode-array detector. The standards used were (±)-α-tocopherol (purity ≥ 97.0%) and retinol (purity > 97.0%) (Fluka/Sigma-Aldrich, Steinheim, Germany). A modified EN 14130 (2004) European standard was used to determine the vitamin C (ascorbic acid and dehydroascorbic acid) content. The feed samples were homogenized, vitamin C was extracted with 2% metaphosphoric acid, the extract was filtered through a 0.22 μm membrane filter, and analyzed by HPLC using a Synergi 4 μm Fusion-RP 80A column (Phenomenex, Torrance, USA) and gradient elution (25mM KH_2PO_4 : acetonitrile). The pH of the 25mM KH_2PO_4 was adjusted to 3 using 20% MPA. L(+)Ascorbic acid puriss (purity ≥ 99.7%) (Sigma-Aldrich, Steinheim, Germany) was used as a standard.

Lipid peroxidation in the yolks (n = 12 + 12 + 12) of fresh eggs and eggs stored for 14 or 28 days at 4°C was measured using the thiobarbituric acid method described by Piette and Raymond (1999). Thiobarbituric acid-reactive substances were expressed as mg of malondialdehyde per kg.

The data were statistically analyzed using Two-Way Analysis of Variance (ANOVA) (main effect of Se, vitamin C, and their interaction) with the General Linear Models (GLM) Procedure of SAS (Statistical Analysis System, Version 8.2, 2003). All differences were considered non-significant at $P > 0.05$. Results in the tables are presented as means and SEM.

RESULTS

The average vitamin C concentration in the supplemented diets was 184.4 and 115.4 mg/kg at the beginning of the experiment and after 12 weeks, respectively. Supplementation of the basal diet with vitamin C or Se significantly increased laying performance (Table 2). The only recorded mortality was the loss of one hen in the control group. In diets supplemented with Se, however,

Table 2. Treatment effects on performance of laying hens

Selenium	–		Na-selenite		Se-yeast		SEM	Probability		
Vitamin C (mg/kg)	0	150	0	150	0	150		vit. C	Se	vit. C × Se
Egg production (g/bird per day)	57.6^{c}	58.4^{b}	59.0^{b}	57.1^{c}	59.9^{a}	58.8^{b}	0.11	< 0.05	< 0.05	< 0.05
Feed intake (g/bird per day)	119.0	118.0	118.0	113.6	118.7	116.1	0.31	< 0.05	ns	ns
Feed intake (kg/kg egg weight)	2.17^{a}	2.07^{b}	2.06^{b}	2.08^{b}	2.06^{b}	2.04^{b}	0.007	< 0.05	< 0.05	< 0.05

$^{a–c}$means in the same row with different superscripts differ significantly; ns = not significant

Table 3. Effects of dietary vitamin C and selenium on parameters of egg quality

Selenium	–		Na-selenite		Se-yeast		SEM	Probability		
Vitamin C (mg/kg)	0	150	0	150	0	150		vit. C	Se	vit. C × Se
Egg weight (g)	62.8	62.8	61.7	63.4	62.7	63.8	0.16	ns	< 0.05	ns
Shell percentage	10.1	10.1	10.3	10.2	10.0	10.1	0.03	ns	ns	ns
Albumen percentage	65.6	65.7	65.3	65.9	65.9	66.0	0.08	ns	ns	ns
Yolk percentage	24.3	24.2	24.5	24.0	24.1	23.9	0.08	ns	ns	ns
Haugh units	87.8	86.7	87.5	88.2	88.4	87.8	0.24	ns	ns	ns
Shell strength (N)	38.65^{b}	40.17^{ab}	40.51^{a}	41.22^{a}	40.27^{a}	39.80^{ab}	0.228	ns	ns	< 0.05
Shell index (g/100 cm^{2})	85.0	85.0	84.6	85.3	85.0	85.5	0.07	ns	< 0.05	ns
Lightness, L^*	57.9	58.1	57.2	57.5	58.3	57.6	0.11	ns	ns	ns
Redness, a^*	15.6	15.4	15.4	15.2	15.5	15.6	0.07	ns	ns	ns
Yellowness, b^*	46.3^{a}	45.2^{b}	44.8^{bc}	44.4^{c}	45.5^{ab}	45.5^{ab}	0.13	< 0.05	ns	< 0.05

$^{a–c}$means in the same row with different superscripts differ significantly; ns = not significant

Table 4. Treatment effects on concentrations of vitamin E, vitamin A, iron, and selenium in eggs

Selenium	–		Na-selenite		Se-yeast		SEM	Probability		
Vitamin C (mg/kg)	0	150	0	150	0	150		vit. C	Se	vit. C × Se
Vitamin E (mg/kg yolk DM)	215.5	240.5	244.9	264.2	255.7	253.4	3.00	< 0.05	< 0.05	ns
Vitamin A (mg/kg yolk DM)	11.8	11.4	12.0	11.6	12.2	11.5	0.11	ns	ns	ns
Iron (µg/kg yolk DM)	137.8^{b}	135.0^{b}	160.8^{a}	140.6^{bd}	144.5^{d}	130.7^{c}	1.41	< 0.05	< 0.05	< 0.05
Selenium (µg/kg yolk DM)	603^{d}	594^{d}	1073^{c}	1117^{b}	1066^{c}	1168^{a}	15.0	< 0.05	< 0.05	< 0.05
Selenium (µg/kg albumen DM)	574^{d}	550^{d}	769^{c}	841^{b}	1545^{a}	1506^{a}	25.1	< 0.05	< 0.05	< 0.05

$^{a–d}$means in the same row with different superscripts differ significantly; ns = not significant, DM = dry matter

Table 5. Treatment effects on concentrations of thiobarbituric acid-reactive substances in fresh yolks (TBARS 0) and yolks stored at 4°C for 14 and 28 days (TBARS 14 and 28)

Selenium	–		Na-selenite		Se-yeast		SEM	Probability		
Vitamin C (mg/kg)	0	150	0	150	0	150		vit. C	Se	vit. C × Se
TBARS 0 (mg/kg)	0.91^{ab}	0.95^{a}	0.81^{c}	0.81^{c}	0.87^{b}	0.90^{ab}	0.008	ns	< 0.05	< 0.05
TBARS 14 (mg/kg)	0.95^{b}	1.01^{a}	0.87^{c}	0.89^{c}	0.92^{b}	0.93^{b}	0.007	< 0.05	< 0.05	< 0.05
TBARS 28 (mg/kg)	0.99^{b}	1.12^{a}	0.93^{b}	0.98^{b}	0.97^{b}	0.97^{b}	0.009	< 0.05	< 0.05	< 0.05

$^{a–c}$means in the same row with different superscripts differ significantly; ns = not significant

vitamin C significantly decreased egg production. The significant interaction between the source of Se and vitamin C was found in shell breaking strength and yolk yellowness (Table 3). The combination of vitamin C and Se increased shell breaking strength and decreased values of yolk yellowness in comparison with the basal diet. Most of the egg quality parameters were not significantly affected. In Se-supplemented hens, vitamin C significantly reduced the concentration of iron in the yolk (Table 4). Selenite, however, had an opposite effect. Vitamin C supplementation significantly increased vitamin E concentration and decreased Fe concentration in the yolks of hens fed diets supplemented with Se. Selenium supplementation significantly increased vitamin E concentration in the yolk and Se concentration in the yolk and albumen. The concentration of vitamin A in the yolk was not influenced by Se or vitamin C addition. The concentration of products from lipid peroxidation (TBARS) increased marginally during the refrigerated storage of eggs. In eggs from hens fed the basal diet, vitamin C supplementation significantly increased the concentration of TBARS in yolks stored for 14 or 28 days (Table 5). The TBARS concentration in yolks of fresh eggs and eggs stored for 14 days was decreased in hens fed diets supplemented with sodium selenite.

DISCUSSION

Stability of the vitamin C preparation used in the present study was limited, as 37.4% was lost in the course of 12 weeks, a typical shelf life for feed mixtures. Vitamin C increased the laying performance of hens, which is consistent with the observations of Peebles and Brake (1985). In most studies, however, vitamin C failed to improve egg production (reviewed by Pardue and Thaxton, 1986). Both vitamin C and Se increased vitamin E deposition in the egg yolk. The sparing effect of Se on the amount of vitamin E in eggs has been described previously (Surai, 2000; Skřivan et al., 2008). Vitamin C can reduce tocopheryl radicals formed in reactions with reactive oxygen species; in our previous experiment, however, no sparing effect of vitamin C on the amount of vitamin E in the meat of broilers was apparent (Skřivan et al., 2012). A moderate increase in the concentration of products of lipid peroxidation measured as TBARS in the yolk was observed in all treatment groups during the refrigerated storage of eggs. The oxidative stability of yolk lipids was improved in hens fed diets supplemented with sodium selenite but not in those fed diets supplemented with Se-yeast. After 28 days of storage, however, the difference between the sources of Se disappeared. Selenium increases lipid stability via the activity of Se-dependent glutathione peroxidase, which is an enzyme that catalyzes the reduction of hydrogen peroxide and organic peroxides (Behne and Kyriakopoulos, 2001; Wang et al., 2011b; Pavlata et al., 2012). Supplementation of the basal diet with vitamin C significantly reduced oxidative stability of yolk lipids, indicating that vitamin C acted as a pro-oxidant. Ascorbic acid, which is a known antioxidant, can in some circumstances act as pro-oxidant, particularly when animals have adequate vitamin E stores and vitamin C is supplemented in large doses (Chen, 1989). Franchini et al. (2002) reported elevated concentrations of TBARS in eggs of hens fed a diet supplemented with vitamin C and vitamin E at 500 and 100 mg/kg, respectively.

Vitamin C increases the absorption of iron from the gut (Mayes, 2002). Among hens fed the basal diet, vitamin C did not influence iron concentration in the yolk but reduced iron deposition when combined with Se. In a previous experiment, the deposition of dietary iron (120 mg/kg) in eggs was low, but it was increased when the combination of iron, copper, and zinc was used (Skřivan et al., 2005).

Sünder and Flachowsky (2001) found that high vitamin E supplementation (1 g/kg) decreased the concentration of canthaxanthin, and at very high vitamin E dosages (10 g/kg), a significant decrease in yolk colour was observed. A reduced deposition of carotenoids, which are responsible for the yolk colour, may be the reason for the colour change in egg yolks of hens fed the basal diet supplemented with vitamin C. The interaction of plant pigments and feed additives with yolk colour merits further research.

Both inorganic and organic sources of Se have been used to increase the Se concentration in poultry products. Organic Se sources (Se-yeast, Se-algae) were more effective in increasing the Se content in eggs than selenite (Skřivan et al., 2008). In the present study, more Se of Se-enriched yeast was deposited in the albumen than in the egg yolk. The incorporation of Se into the yolk proteins was not influenced by its source.

CONCLUSION

Effects of vitamin C supplementation in diets of laying hens were equivocal. Supplementation of the basal wheat-maize-soybean diet with vitamin C and Se or the combination of vitamin C and Se-yeast increased the performance of hens, however, the combination of vitamin C and sodium selenite decreased the eggs production.

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