

EFFECTS OF SELENIUM SUPPLEMENTATION AS SODIUM SELENITE OR SELENIZED YEAST AND DIFFERENT AMOUNTS OF VITAMIN E ON SELENIUM AND VITAMIN E STATUS OF BROILERS

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The aim of this experiment was to determine the effects of broiler meal supplementation with different forms of selenium (as Na-selenite or selenized yeast) and different amounts of vitamin E on selenium and vitamin E status in broiler tissues.

A total number of 240 broilers (Cobb 500) were divided in four experimental groups supplemented with Se and vitamin E for a period of 42 days: group SS+E20 – 0.3 mg/kg sodium selenite and 20 IU of vitamin E; group SY+E20 – 0.3 mg/kg selenized yeast and 20 IU of vitamin E; group SS+E100 – 0.3 mg/kg sodium selenite and 100 IU of vitamin E; group SY+E100 – 0.3 mg/kg selenized yeast and 100 IU of vitamin E. Blood plasma Se and MDA concentrations and Se dependent GSH-Px were determined on days 1, 21 and 42, whereas content of Se and vitamin E in breast muscle and liver were measured on days 21 and 42 of the experiment. Highest blood plasma glutathione peroxidase (GSH-Px) activities were detected in groups SS+E20 and SS+E100. Supplementation with selenium enriched yeast did not result in a significant increase in plasma GSH-Px activity.

Selenium and vitamin E concentrations in breast meat and liver were significantly higher in groups supplemented selenized yeast compared to those receiving Na-selenite. Selenium and vitamin E supplementation did not alter plasma MDA concentrations, but in tissues, selenized yeast provided a consistent, although not significant, reduction in MDA content. The increased dose of vitamin E supplemented in broiler meal was not justifiable on the basis of vitamin E tissue content and antioxidative effect.

Key words: sodium selenite, selenized yeast, selenium, vitamin E, broilers

INTRODUCTION

To achieve adequate growth and health of broilers they should be provided with sufficient amounts of all necessary ingredients, including antioxidative compounds selenium and vitamin E.

In synergetic action, Se and vitamin E prevent the widespread chain reaction of fatty acid peroxidation in animal tissues. The biological role of selenium as a part of the antioxidative defence system is expressed through glutathione peroxidase (GSH-Px; EC. 1.11.1.9) of the selenoenzyme family (Behne and Kyriakopoulos, 2001). As other selenoproteins GSH-Px contains selenium in its active site in the form of selenocysteine. Together with enzymes catalase and superoxide dismutase, GSH-Px prevents formation and auto-propagation of free radicals by reducing peroxides to alcohols (Ganther, 1975). The role of vitamin E is to break the chain autooxidation reaction of polyunsaturated fatty acid (PUFA) in cell membranes.

It is well established that the expression and the activity of GSH-Px are dependant on adequate alimentary supply of Se. Serbia is considered to be a moderately selenium deficient territory with significant regional variations in foods and feeds ranging from 20 to 70 µg Se/kg (Mihailović *et al.*, 1996; Jovanović *et al.*, 1998). Therefore, The Animal Feed Rulebook (Službeni list 2000/20) prescribes mandatory supplementation of chicken and turkey broilers with 0.15 mg Se/kg. Safe selenium supplementation of animals for meat production is indicated not only as a mean to sustain good production and meat quality, but also to fortify the human food chain with this microelement.

Daily selenium requirement during intensive broiler growth is 0.15 mg/kg (NRC, 1994). Maximum allowed content of selenium into feed mixtures is 0.30 mg/kg (FDA, 2000). When supplementing animals with inorganic selenium (most often sodium selenite) there is the possibility of intoxication, since inorganic forms of selenium, especially selenates, are more toxic than organic forms (Spallholz, 1994).

Two forms of selenium are used as feed supplements. Inorganic forms, usually selenites and selenates have a history of almost 60 years in animal nutrition (Combs and Combs, 1986). Part of this history includes events of accidental poisoning of both animals and men due to miscalculations and high toxicity of inorganic selenium forms (Mihailović, 1996). Natural animal feeds, including selenized yeast, contain organic forms of selenium, mostly as selenomethionine (Surai and Dvorska, 2002). However, all relevant animal selenoproteins contain selenocysteine (SeCys) as the physiologically active component. Therefore, in recent years the interest raised for the use of selenium enriched yeast as an efficient direct source of selenomethionine in animal feed mixtures (Edens, 2001) which can be stored in muscles indiscriminately with methionine and subsequently converted to selenocysteine and incorporated in selenoproteins (Finley, 2006).

Recommended concentration of vitamin E in broiler mixtures is 15-20 mg/kg of feed. Numerous studies have shown that usage of high amounts of vitamin E presents an effective way to improve quality and sustainability of broiler meat (Jensen *et al.*, 1998).

The purpose of this article is to evaluate the influences of selenium supplement sources (as Na-selenite or selenized yeast) to the selenium status of broilers in the conditions of overall selenium abundancy in feed, as well as

mutual interdependency of Se and vitamin E as elements of the antioxidant defence system.

MATERIAL AND METHODS

A total number of 240 unsexed, one-day chicks (Cobb 500) were used. Initial average body weight was 41.25 ± 2.97 g. During the trial technological conditions were in full compliance with the prescribed norms (Cobb broiler management guide, 2004).

Broilers were fed complete mixtures (FSH "Proteinka" Šabac) of standard chemical composition. Three types of mixtures were used (Table 1), adjusted to the needs broilers in different growth stages (NRC, 1994).

Table 1. Raw and chemical composition of broiler feed mixtures %

Feeds	% of mixture		
	1	2	3
Corn grain	48.82	58.20	64.10
Soybean meal, 44%	11.50	8.00	–
Fulfat soybean meal, 35%	25.60	20.00	22.50
Sunflower meal, 40%	10.00	10.00	10.00
Methionine	0.13	0.05	0.10
Salt	0.30	0.30	0.30
Monocalciumphosphate	0.80	0.75	0.40
Lime	1.65	1.50	1.40
Minazel P plus	0.20	0.20	0.20
VMD	1.00	1.00	1.00
Σ	100.00	100.00	100.00

Chicks were divided in to four experimental groups supplemented with different forms of selenium and different ammounts of vitamin E (Table 2).

Selenium was supplemented as sodium selenite (Microgran™ Se 1% BMP, DSM Nutritional Products, Switzerland) containing 10 mg/kg selenium, or selenium enriched yeast (Sel-Plex 2000, Alltech Inc®, USA) containing 2.000 mg/kg selenium. Selenium content in base feeds prior to supplementation, was 180 mg/kg Se in all three mixtures.

Vitamin E was supplemented in the form of alfa-tocopherol acetate (Rovimix® E-50 Adsorbate, DSM Nutritional Products, Switzerland) containing 500 IU of vitamin E/g.

Table 2. Content of selenium and vitamin E in mixtures

Group	Selenium, [mg/kg] feed DM		Vitamin E, [IU]
	Sodium selenite	Sel-Plex	
SS+E20	0.3	–	20
SY+E20	–	0.3	20
SS+E100	0.3	–	100
SY+E100	–	0.3	100

Heparinized blood samples were taken by cardiac puncture on days 1, 21 and 42 of the experiment, from 6 chicks of each group, and used to determine plasma GSH-Px activity, Se and malondialdehyde (MDA) concentrations. At days 21 and 42, 6 chicks from each group were sacrificed, and samples of breast muscles and liver, were collected for determination of Se and vitamin E content in these tissues.

Activity of plasma glutathione peroxidase (GSH-Px) was measured by coupled test (Günzler *et al.*, 1974) at 366 nm using Cecil CE2021 spectrophotometer with a Peltier thermostat unit set at 37°C, using tetra-bythil-hidroperoxide (TBH) as substrate. Measurement principle was based on spectrophotometric measuring of NADPH utilization in a coupled enzyme system using reduced glutathione (GSH) as immediate GSH-Px reducing co-substrate. Low concentration of TBH <2.32 mmol ensured that only the selenium dependant GSH-Px activity was recorded (Burk *et al.*, 1978).

Tissue MDA concentrations were determined spectrophotometrically at 535 nm. Preparation of blood plasma samples was preformed by the method described by Andreeva *et al.* (1988). Muscle and liver samples were prepared using the method of Uchijama (1978).

Determination of tissue selenium content was performed on ICP/MS ELAN DRC (PERKIN ELMER) according to appropriate calibration curve (EPA 846 3015).

Vitamin E determination was done on HPLC SUMMIT (DIONEX) with auto sampler (JUS ISO 6867/2004).

Statistical analysis of intergroup differences of means was performed using ANOVA, Tukey test and student's t-test, at significance levels of 1 and 5% (Snedecor and Cochran 1971). Software package PrismaPad v.4.0 was used for statistical calculation.

RESULTS AND DISCUSSION

Selenoenzyme GSH-Px activity in blood plasma did not differ significantly throughout the experiment. Highest activities were recorded (Tab. 3) in group SS+E20 days 21 ($125.33 \pm 38.80 \mu\text{kat/L}$) and 42 ($147.33 \pm 33.95 \mu\text{kat/L}$). Pešut (2005a) feeding Hybro broilers with Se supplemented of 0.3 mg/kg as selenized

yeast, after 28 days measured plasma GSH-Px activities ranging from 40-50 μ kat/L and significantly lower activities, from 30-40 μ kat/L in those fed 0.3 mg/kg Na-selenite.

Table 3. Blood plasma glutathione peroxidase (GSH-Px) activity in broilers (μ kat/L)

Group	Day	n	Plasma GSH-Px	
			M \pm SD	CV %
All groups	1	10	109.21 \pm 28.04 ^{a,b}	25.67
SS+E20	21	6	152.77 \pm 38.80 ^a	25.40
SY+E20		6	121.20 \pm 23.97	19.78
SS+E100		6	123.17 \pm 32.35	26.11
SY+E100		6	132.19 \pm 34.07	25.77
SS+E20	42	6	147.33 \pm 33.95 ^b	23.04
SY+E20		6	130.54 \pm 20.75	15.89
SS+E100		6	114.51 \pm 21.26	18.57
SY+E100		6	127.76 \pm 19.33	15.13

^{a,b}p<0.05

Plasma Se concentration in 21 day chicken was significantly higher only in the group SY+E100 compared to day 1 (Table 4). Groups receiving Se supplemented in the form of selenized yeast had higher plasma Se concentrations compared to those receiving Na-selenite, although the difference could not be statistically verified. Day 42 plasma Se concentrations, of all groups were significantly higher compared to day 1 without marked differences between Se supplements.

Supplementing broilers (Ross \times Ross) with 0.30 mg/kg Se as Na-selenite or selenized yeast for 50 days, Payne and Southern (2005) detected Se concentrations in plasma of 137 and 160 μ g/kg respectively, similar to our findings. However, they detected much higher Se content in the breast muscles i.e. 545 and 1170 μ g/kg, respectively.

Highest Se content in breast muscle was detected in the group SY+E100 day 21 (340 \pm 24.49 μ g/kg) and day 42 (426.70 \pm 40.82 μ g/kg). In each of the groups selenized yeast proved to be a significantly more efficient Se source compared to Na-selenite (Table 5, Graph 1a). Day 42 groups receiving 100 IU vitamin E reached significantly higher Se levels compared to those receiving 20 IU, indicating the functional interdependency of these two antioxidant factors (Surai and Dvorska, 2002).

Table 4. Blood plasma selenium concentration in broilers ($\mu\text{g/L}$)

Group	Day	n	Plasma Se	
			M \pm SD	CV %
All groups	1	10	146.67 \pm 23.69 ^{A,a,b,c,d}	16.16
SS+E20	21	6	143.80 \pm 25.59	17.79
SY+E20		6	170.00 \pm 50.00	29.45
SS+E100		6	147.70 \pm 44.34	30.03
SY+E100		6	185.30 \pm 39.44 ^a	21.28
All groups		24	161.70 \pm 41.90 ^e	25.91
SS+E20	42	6	180.70 \pm 34.22 ^b	18.94
SY+E20		6	197.30 \pm 40.78 ^A	20.67
SS+E100		6	194.00 \pm 47.16 ^c	24.31
SY+E100		6	202.20 \pm 60.79 ^d	30.07
All groups		24	193.50 \pm 44.40 ^e	22.95

^A $p < 0.01$; ^{a-e} $p < 0.05$

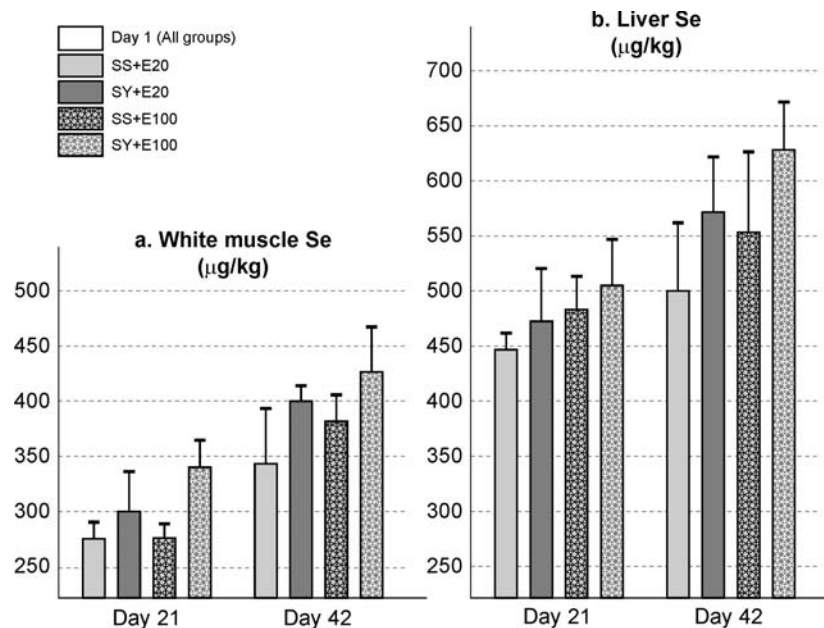
Table 5. Breast muscle and liver selenium contents in broilers ($\mu\text{g/kg}$)

Group	Day	n	Breast muscle Se		Liver Se	
			M \pm SD	CV %	M \pm SD	CV %
SS+E20	21	6	275.00 \pm 15.17 ^B	5.51	446.70 \pm 15.06 ^d	3.37
SY+E20		6	300.00 \pm 36.19 ^{C,a}	12.06	472.50 \pm 47.93	10.14
SS+E100		6	275.80 \pm 12.81 ^{A,b}	4.65	483.30 \pm 30.11	6.23
SY+E100		6	340.00 \pm 24.49 ^{A,E,a}	7.20	505.00 \pm 40.87 ^d	8.09
All groups		24	297.70 \pm 35.00 ^F	11.76	476.90 \pm 39.60 ^G	8.30
SS+E20	42	6	343.30 \pm 50.07 ^{B,b}	14.58	500.00 \pm 61.97 ^H	12.39
SY+E20		6	400.00 \pm 14.14 ^{C,b}	3.54	571.70 \pm 49.97	8.74
SS+E100		6	381.70 \pm 24.01 ^{D,c}	6.29	553.30 \pm 74.48	13.46
SY+E100		6	426.70 \pm 40.82 ^{E,c}	9.57	628.30 \pm 43.55 ^H	6.93
All groups		24	387.90 \pm 45.10 ^F	11.63	563.30 \pm 72.00 ^G	12.78

^{A-H} $p < 0.01$; ^{a-d} $p < 0.05$

Selenium content in all groups in the liver was significantly higher compared to white muscle tissue on days 21 and 42. Again, selenized yeast yielded higher

liver Se content than Na-selenite, but statistical significance could not be established, as it is shown in Graph 1b.



Graph 1. a. Breast muscle and b. liver Se contents in broilers (µg/kg)

In trials similar to ours, Arnold *et al.* (1974) using vitamin E supplement (10 IU) and inorganic selenium (0, 0.1 and 1.0 mg/kg) obtained selenium content in broiler breast muscles ranging from 0.37 to 0.44 mg/kg and in liver from 0.67 to 0.72 mg/kg. However, Pešut *et al.* (2005b) by supplementing with 0.3 mg/kg Se as sodium selenite obtained a content of 0.45 mg/kg in liver and in breast muscle 0.09 mg/kg, and using selenized yeast 0.43 mg/kg in liver and 0.19 mg/kg breast muscle.

Overall tissue Se contents clearly followed the well established pattern for most animal species: blood plasma < muscle < liver (Surai, 2002). Since the content of Se in muscle and liver exhibited a steady growth until the end of experiment without the tendency to plateau, it can be concluded that these tissues could be a good and safe source of Se for human consumption, given that broilers are properly supplemented.

Highest plasma vitamin E concentration (Table 6) was detected in one-day-old chicks (5.10 ± 0.57 µg/mL), which subsequently fell. A similar pattern was detected by Surai, 1999. Day 21 groups fed selenized yeast had significantly higher plasma vitamin E concentration compared to corresponding groups fed sodium selenite, while on day 42, levels equaled at 4 µg/mL. Arslan *et al.* (2000)

obtained by feeding broilers 100 UI of vitamin E after 5 and 7 weeks plasma concentrations of 2.41 and 2.42 µg/mL, slightly below the concentrations we had measured.

Table 6. Blood plasma vitamin E concentration in broilers (µg/mL)

Group	Day	n	Plasma vitamin E	
			M ± SD	CV %
All groups	1	10	5.10 ± 0.57	11.17
SS+E20	21	6	2.79 ± 0.07 ^{A,a}	2.38
SY+E20		6	3.53 ± 0.48 ^{A,b}	13.71
SS+E100		6	2.92 ± 0.25 ^b	8.51
SY+E100		6	3.43 ± 0.43 ^a	12.45
SS+E20	42	6	3.73 ± 0.70	18.84
SY+E20		6	3.85 ± 0.69	17.83
SS+E100		6	3.87 ± 0.59	15.31
SY+E100		6	4.08 ± 0.56	13.72

^A p<0.01; ^{a, b} p<0.05

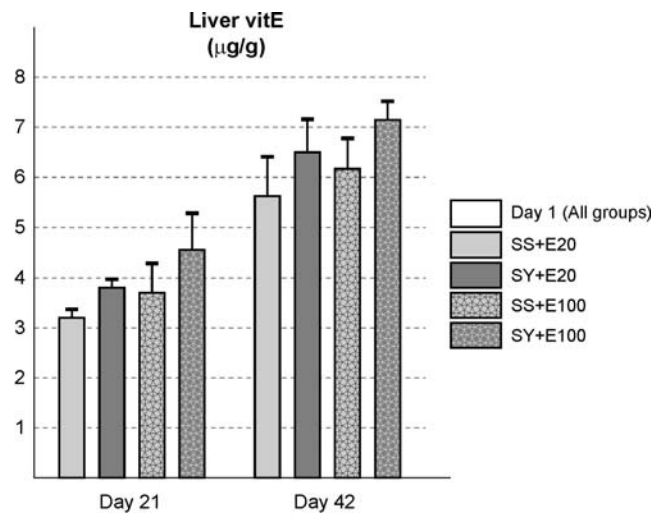
Overall breast muscle vitamin E content was lower compared to corresponding values in plasma and did not rise during the experiment, except for the group SY+E100 day 42 (Table 7), which exhibited the highest vitamin E content (3.88 ± 0.43 µg/g). Surai and Dvorska (2002) using supplementation of 0.20 mg/kg of selenium (Sel-Plex) plus 40 IU vitamin E measured vitamin E concentration in breast muscle to be 3.22 ± 0.36 µg/g and when using 0.4 ppm of selenium plus 100 IU increased to 5.88 ± 0.59 µg/g.

Liver vitamin E content raised steadily and significantly throughout the experimental period and almost doubled on day 42 compared to day 21 (Table 7, Graph 2) reconfirming the role of liver tissue as body vitamin E storage. It is interesting to notice that in many cases vitamin E content was significantly higher in groups receiving Se supplemented as selenized yeast compared to those receiving Na-selenite. It indicates that organified selenium, whether in the form of selenocysteine, or some other selenium compound, might have some influence to vitamin E metabolism in poultry tissues (Surai and Dvorska, 2002). It is also clearly visible (Tables 6 and 7) that high doses of vitamin E of 100 IU had negligible influence to its tissue contents compared to groups supplemented with 20 IU, probably due to efficient elimination of excess vitamin E from the body.

Table 7. Breast muscle and liver vitamin E contents in broilers ($\mu\text{g/g}$)

Group	Day	n	Breast muscle vit. E		Liver vit. E	
			M \pm SD	CV %	M \pm SD	CV %
SS+E20	21	6	2.08 \pm 0.26 ^{A,B}	12.36	3.20 \pm 0.17 ^E	5.23
SY+E20		6	2.68 \pm 0.19 ^A	7.23	3.80 \pm 0.17	4.40
SS+E100		6	2.48 \pm 0.22	9.01	3.70 \pm 0.59 ^a	16.04
SY+E100		6	2.85 \pm 0.40 ^B	13.99	4.55 \pm 0.73 ^{E,a}	16.14
All groups		24	2.52 \pm 0.39 ^D	15.48	3.81 \pm 0.67 ^G	17.59
SS+E20	42	6	2.62 \pm 0.38 ^A	14.38	5.62 \pm 0.79 ^F	14.08
SY+E20		6	3.00 \pm 0.34 ^B	11.35	6.50 \pm 0.66	10.20
SS+E100		6	2.90 \pm 0.23 ^C	7.86	6.17 \pm 0.61	9.87
SY+E100		6	3.88 \pm 0.43 ^{A,B,C}	10.98	7.15 \pm 0.37 ^F	5.21
All groups		24	3.10 \pm 0.58 ^D	18.71	6.36 \pm 0.81 ^G	12.74

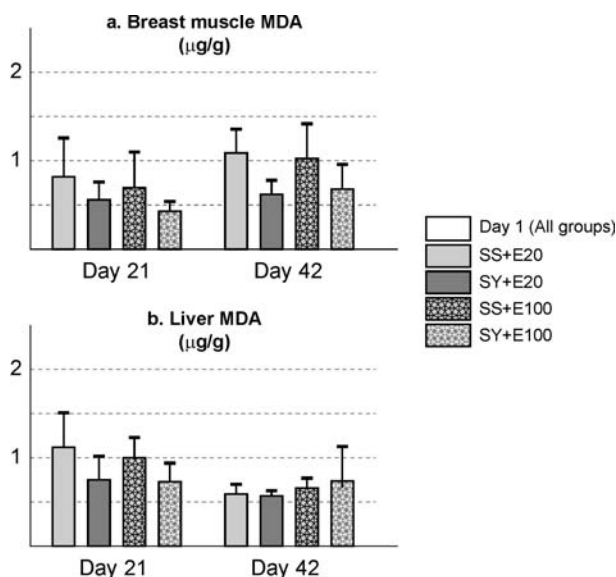
A-G $p < 0.01$; a $p < 0.05$



Graph 2. Liver vitamin E contents in broilers ($\mu\text{g/g}$)

Initial MDA concentration in one-day-old chicks was $7.43 \pm 0.98 \mu\text{M}$ and subsequently slightly, but nonsignificantly decreased towards the end of the experiment. Therefore, it is possible to speculate that bulk plasma MDA is

produced in tissues during the oxidative metabolism and transported in the plasma, hence plasma antioxidative systems can not have significant impact on it's concentration.



Graph 3. a. Breast muscle and b. liver vitamin E contents in broiles

MDA contents in breast muscle and liver tissues were low, ranging from 0.57 ± 0.06 to 1.12 ± 0.39 mg/kg. These concentrations are in accordance with results obtained by Surai and Dvorska (2002), Pešut (2005). Groups receiving Se supplement in the form of selenized yeast consistently exhibited lower MDA concentrations in breast muscle throughout the whole experiment and in the liver on day 21, although significance could not be established due to high variability, except for group SY+20E ($p < 0.01$ compared to SS+20E). High vitamin E doses of 100 IU did not reduce tissue MDA contents below levels measured in groups supplemented with 20 IU. This finding is in conjunction with the fact that such high doses do not stockpile vitamin E more efficiently suggests that addition of 100 IU vitamin E to broiler feed might not be physiologically or economically justified.

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UTICAJ DODAVANJA SELENA ORGANSKOG I NEORGANSKOG POREKLA I RAZLIČITIH KOLIČINA VITAMINA E NA STATUS SELENA I VITAMINA E BROJERA

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SADRŽAJ

Cilj ovog eksperimenta je bio da se utvrde efekti dodatka različitih formi selena u obrok (natrijum selenit ili selenizirani kvasac) i različitih količina vitamina E na status selena i vitamina E u tkivima brojlera.

Ukupan broj od 240 brojlera (Cobb 500) je bio podeljen na četiri eksperimentalne grupe kojima je dodavan selen i vitamin E u obrok u periodu od 42 dana: grupa SS+E20 - 0,3 mg/kg Na-selenita i 20 IJ vitamina E; grupa SY+E20 - 0,3 mg/kg seleniziranog kvasca i 20 IJ vitamina E; grupa SS+E100 - 0,3 mg/kg Na-selenita i 100 IJ vitamina E; grupa SY+E100- 0,3 mg/kg seleniziranog kvasca i 100 IJ vitamina E. Koncentracija selena u krvnoj plazmi i MDA kao i selen zavisne GSH-PX je određivana 1., 21. i 42., dok su sadržaj selena i vitamina E u grudnoj muskulaturi i jetri određivani 21. i 42. dana eksperimenta. Najviše vrednosti aktivnosti glutation peroksidaze u krvnoj plazmi su utvrđene u grupama SS+E20 i SS+E100. Dodatak seleniziranog kvasca nije rezultirao značajnim povećanjem aktivnosti GSH-Px u krvnoj plazmi.

Koncentracija selena i vitamina E u grudnoj muskulaturi i jetri je bila značajno viša u grupama kojima je dodat selenizirani kvasac u poređenju sa grupama koje su dobijale Na-selenit. Dodatak selena i vitamina E nije uticao na koncentraciju MDA u krvnoj plazmi, ali je u tkivima, selenizirani kvasac doveo do uočljivog ali ne značajnog smanjenja sadržaja MDA. Povećana doza vitamina E u obroku brojlera od 100 IJ vitamina E nije bila opravdana na osnovu sadržaja vitamina E i antioksidativnog dejstva.