

EFFECT OF SELENIUM-FORTIFIED WHEAT IN FEED FOR LAYING HENS ON TABLE EGGS QUALITY

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Abstract

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The aim of the study was to determine the effect of selenium-fortified wheat added in feed for laying hens on egg quality and content of selenium in the edible part of the egg. The study was conducted on 105 hens of TetraSL hybrid which were in a 40th week of production. Laying hens were divided into three groups. Group A was control, and B and C experimental in which 10% of the corn in a mixture was replaced with selenium-fortified wheat. Analysis of eggs stored for three days showed that the used treatments significantly influenced the mass of eggs and basic parts of eggs. Eggs from groups A and C had significantly higher values of length, width and eggshell share compared to eggs from group B. Group A eggs had a significantly thicker shell and more intensive yolk color compared to eggs from groups B and C. The impact of treatment on yolk color was determined for eggs stored for 28 days. Lipid oxidation in yolks of eggs stored for 3 or 28 days was equable among all three experimental groups. It was found that the selenium content in the albumen of the egg was influenced by treatments and time of analysis of selenium. Usage of selenium-fortified wheat in the diets for laying hens may have an influence on a better supply of this microelement in the edible part of the egg, with optimal external and internal egg quality indicators.

Key words: egg quality, fortified wheat, laying hens, selenium, TBARS

Abbreviations: TBARS – thiobarbituric acid reactive substances; HU – Haugh units

Introduction

In animal feeding selenium is supplemented in two forms, inorganic and organic. Selenium is an essential microelement. People and animals must take selenium with food. The role of selenium in the body is multiple. Selenium is an irreplaceable part of a series of biochemical processes in the body. Many scientists whose researches were focused on the impact of the selenium source on its availability in the body of animals, in their research results point out a better bioavailability of selenium if it is added in organic form in the feed for animals (Rayman, 2004; Payne et al., 2005; Skrivan et al., 2006). Recently, in crop production there is a tendency to increase the content of various microelements in grains through fortifica-

tion. Animals will then consume those microelements in their meal in organic form (Hassan, 1990; Haug et al., 2008).

The aim of this study was to determine the effect of using selenium-fortified wheat in feed for laying hens on the egg quality and content of selenium in the edible part of the eggs.

Materials and Methods

The study was conducted on 105 Tetra SL hybrid hens, divided into three groups (A, B and C). Hens were in the 40th week of production and experimental period lasted for 26 days. During the experimental period hens were consuming mixtures in which 10% of corn was replaced with selenium-fortified wheat of Srpanjka variety, which was grown on calcareous soils with

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7 different treatments in a randomized block design. For feeding experiments wheat from three fertilization treatments was used, as follows: A = control without Se, B = foliar application of Se with 5 g Se ha⁻¹ and C = foliar application of Se with 10 g Se ha⁻¹. The composition of mixtures for hens is shown in Table 1. Before starting the experiment hens were weighed and randomly divided into three experimental groups. Analysis of selenium content in mixtures was made shortly before feeding trial. It was determined that mixture A contains 0.2522 mg Se/kg diet, mixture B 0.3059 mg Se/kg diet and mixture C 0.5484 mg Se/kg diet. In the middle of the trial period (day 14) 10 eggs from each group were taken for determination of the concentration of selenium in egg albumens and yolks. At the end of the experimental period eggs were sampled for analysis of egg quality and determination of the content of selenium in the edible part (egg albumen and yolk). There was a total of 150 eggs taken in the last three days of the experiment for different analyses (120 to analyze the external and internal egg quality and TBARS and 30 for analysis of selenium). Eggs which were used for the quality analysis were from class L where belong eggs from 63 g to 73 g of weight. Among external egg quality indicators, following were analyzed: egg weight and egg shape index, eggshell strength, thickness and weight. Considering internal egg quality indicators, analyzed were: weight of egg albumen and yolk, yolk color, albumen height, Haugh units (HU) and pH value of albumen and yolk. Shares of major parts in eggs were also calculated. Egg quality and yolk lipid oxidation were analyzed on eggs stored for 3 or 28 days in the refrigerator at +4°C. The weight of eggs and the basic parts (egg albumen, egg yolk and shell) was determined by electronic scale PB 1502-S. Eggshell strength was measured using automatic device Eggshell Force Gauge Model-II. The thickness of the shell was measured at the middle of the egg shell using an electronic micrometer with an accuracy of 0.001 mm. Shape index was calculated from the measures of egg width and length according to the following pattern: shape index (%) = width of the egg/egg length*100 (Panda, 1996). The color of the yolk, albumen height and HU were determined by automatic device Egg Multi-Tester EMT-5200. pH valueS of albumen and yolk were measured with a pH meter MP 120. Lipid oxidation was measured on 36 yolks (18 fresh and 18 stored) according to the modified method of McDonald and Hultin (1987) and Botsoglou et al. (1994). To the yolk sample weighed in a test tube a 10% trichloroacetic acid was added (1w:3v), the mixture is then homogenized and centrifuged at 10000 rpm for 10 minutes at 4°C. The supernatant is then mixed with a solution of thiobarbituric acid, the tubes were sealed and placed in a water bath at 90°C for 30 minutes. After cooling, distilled water was added and the mixture was centrifuged at 6000 rpm for 5 minutes at 4°C. The content of the colored product resulting from the reaction of products of

lipid peroxidation with thiobarbituric acid was measured spectrophotometrically at 534 nm. Concentration of selenium in egg albumen and yolks was analyzed using a Perkin Elmer Optima device 2100 DV (Davidowski, 1993). The research results were processed using statistical program Statistica for Windows version 12.0 (StatSoft Inc., 2013). Results were processed using

Table 1.

Composition and chemical analysis of mixtures for hens

Ingredient, %	¹ A, B, C
Corn	40.75
Triticale	6.60
Wheat	10.00
Soybean meal	18.33
Toasted soy	8.33
Sunflower meal	1.66
Alfalfa	1.00
Calcium granules	8.13
Monocalcium	1.58
Yeast	0.50
Salt	0.33
Mineral Detox	0.25
Probiotic Pro Bio	0.05
Methionine	0.25
² Premix	0.58
Soybean oil	1.66
Total	100.00
³ Chemical analysis of mixtures (g/kg)	
Moisture	87
Crude protein	190
Crude fiber	40
Ash	139
Fat	52
Ca	41

¹ In mixtures 10% of corn was replaced with wheat: A = control without fortification, B = wheat fortified with selenium 5 g Se ha⁻¹ and C = wheat fortified with selenium 10 g Se ha⁻¹

²Premix mixture K, content in 1 kg: vitamin A 200000 UI, vitamin D₃ 500000 UI, vitamin E 10000 mg, vitamin K₃ 600 mg, vitamin B₁ 400 mg, vitamin B₂ 1000 mg, vitamin B₆ 1000 mg, vitamin B₁₂ 3000 µg, vitamin C 4000 mg, vitamin H 12 mg, vitamin B₃ 8000 mg, vitamin B₅ 2400 mg, vitamin B₉ 150 mg, vitamin B₄ 100000 mg, iodine 200 mg, manganese 18000 mg, zinc 14000 mg, cobalt 30 mg, iron 12000 mg, copper 1600 mg, selenium inorganic 50 mg, calcium 238 g, phytase 100000 FYT, canthaxanthin 500 mg, beta-apo-beta-carotenoic acid 300 mg, antioxidant (butylhydroxy toluene) 20000 mg

³ Reference methods used at the chemical analysis of food: HRN ISO 6496:200; HRN EN ISO 5983-2:2010; HRN EN ISO 6865:2001, Mod. according to instructions of FOSS Fiber Cap manual; HRN ISO 5984:2004; HRN ISO 6492:2001, Mod. according to instructions of the extraction system ANKOM XT15; RU-5.4.2-11 (internal method)

one-factorial or multi-factorial analysis of variance (ANOVA). If the p value was statistically significant, differences between groups were tested by Fisher's LSD test (Table 1).

Results and Discussion

Table 2 shows the weight of eggs and the basic parts of eggs stored for 3 days in a refrigerator at +4°C. It is evident that the treatments used have statistically significant effect on the weight of egg and basic parts of eggs ($p < 0.05$). A control group of hens had significantly higher egg weight in relation to the groups of hens where a corn in a mixture was replaced with fortified wheat (A = 68.88 g in relation to B = 66.10 g and C = 65.95 g; $p = 0.002$). Values for weight of basic parts of eggs ranged in accordance with the values for the egg weight, meaning that a significantly higher weight of albumen (A = 42.31 g, B = 40.85 g and C = 40.77 g; $p = 0.044$) had a control group of hens in relation to the experimental groups. A significantly higher yolk weight was recorded in a group A (17.79 g) in relation to the group C (16.69 g), while the shell weight was significantly higher in group A and C (8.73 g and 8.44 g) in relation to the group B (8.05 g; $p < 0.001$). Gjorgovska et al. (2012) reported that levels of selenium in a meal had a statistically significant effect on weight of eggs and basic parts (albumen, yolk and shell), that is, with increasing levels of selenium in diet, the weight of the eggs and the basic parts in eggs also increases. Their results are not consistent with our results.

Table 2

Weight of eggs and basic parts of eggs stored for 3 days in a refrigerator at +4°C (g; $\bar{x} \pm s$)

Indicators	A	B	C	p value
Egg weight	68.83±3.33 ^a	66.10±1.94 ^b	65.92±2.95 ^b	0.002
Albumen weight	42.31±2.28 ^a	40.85±1.69 ^b	40.77±2.09 ^b	0.044
Yolk weight	17.79±1.15 ^a	17.18±0.99 ^{ab}	16.69±1.51 ^b	0.025
Shell weight	8.73±0.51 ^a	8.05±0.54 ^b	8.44±0.34 ^a	<0,001

\bar{x} = arithmetic mean; s = standard deviation; The numbers in the rows marked with ^{ab} exponents are mutually statistically different ($P < 0.05$; $P < 0.01$ and $P < 0.001$).

Table 3 shows the weight of eggs and the basic parts of the eggs stored for 28 days in a refrigerator at +4°C. From the presented results it is clear that the treatments used in the experiment did not have a statistically significant effect on weight of eggs and basic parts in eggs ($p < 0.05$). Looking at the results of the analysis of eggs after 3 days of storage and after 28 days of storage it is obvious that the weight of the eggs and albumen decreases.

Addition of organic selenium in feed for laying hens, in ad-

Table 3

Weight of eggs and basic parts of eggs stored for 28 days in a refrigerator at +4°C (g; $\bar{x} \pm s$)

Indicators	A	B	C	p value
Egg weight	66.21±2.98	66.02±2.11	64.63±1.84	0.079
Albumen weight	40.05±2.21	39.97±1.78	39.23±1.38	0.295
Yolk weight	17.78±1.05	17.62±0.90	17.19±1.21	0.202
Shell weight	8.37±0.46	8.42±0.55	8.21±0.46	0.369

\bar{x} = arithmetic mean; s = standard deviation

dition to increasing the selenium content in the edible part of the egg, has a positive effect on the freshness of eggs which is associated with quality. The freshness of egg is affected by storage time (in days) and the conditions under which the eggs are kept (temperature and relative humidity). Age of eggs is counted from the moment when the egg is laid until the time of its use. Indicators that are most often used to describe the freshness of eggs are height of air chamber, the pH of egg albumen and yolk, the intensity of lipid oxidation, albumen height and the values of HU. Tables 4 and 5 show the results of the effect of treatment on indicators of external and internal quality of eggs kept in the refrigerator for different period of time. For eggs kept three days in the refrigerator, it is evident that the level of selenium in feed has an effect on the length and width of eggs, meaning that groups A and C had a significantly higher value of these indicators in relation to group B. Shape index was equable in all experimental groups (A = 76.16%; B = 77.09% and C = 76.71%; $p = 0.240$). The values of the eggshell strength and thickness had the same trend. The maximum value for the strength was measured in group C (3.46 kg/cm²), followed by group A (3.28 kg/cm²) and B (3.19 kg/cm²; $p = 0.380$). The thickness of the shell was significantly different between the examined groups (A = 0.437 mm > C = 0.413 mm > B = 0.385 mm; $p < 0.001$). Significantly more intense color of the yolk was determined in experimental group A (12,35) in relation to the groups B and C (11.85 and 11.60; $p = 0.010$). Although the difference for the albumen height and HU between the groups was not statistically significant ($p = 0.250$ and $p = 0.317$), the best values for these indicators were found in group C (6.82 mm and 80.21) followed by group B (6.72 and 77.10 mm) and group A (6.29 mm and 75.01). The pH values of albumen and yolk in all groups were uniform ($p > 0.05$).

For eggs kept in the refrigerator for 28 days, it can be noticed that the level of selenium in the feed has an effect on the yolk color ($p = 0.029$), meaning that eggs from group A have a more intense color of the yolk relative to eggs from group C (12.45 and 11.85). For other analyzed indicators of external and internal egg quality, resulting differences

Table 4**Effect of treatments on indicators of external and internal quality of eggs stored for 3 days at 4°C ($\bar{x} \pm s$)**

Indicators	A	B	C	p value
Egg length, mm	59.55±1.61 ^a	57.35±1.69 ^b	58.55±1.82 ^a	<0.001
Egg width, mm	45.35±1.08 ^a	44.20±1.10 ^b	44.90±1.29 ^a	0.010
Shape index, %	76.17±1.66	77.09±1.73	76.71±1.73	0.240
Eggshell strength, kg/cm ²	3.28±0.79	3.19±0.45	3.46±0.62	0.380
Eggshell thickness, mm	0.437±0.02 ^a	0.385±0.02 ^c	0.413±0.02 ^b	<0.001
Yolk color	12.35±0.58 ^a	11.85±0.87 ^b	11.60±0.82 ^b	0.010
HU	75.01±9.15	77.10±14.97	80.21±6.52	0.317
Albumen height, mm	6.29±1.20	6.72±0.96	6.82±0.94	0.250
Albumen pH	8.76±0.12	8.73±0.11	8.75±0.09	0.605
Yolk pH	6.08±0.66	5.98±0.04	6.13±0.066	0.668

\bar{x} = arithmetic mean; s = standard deviation; The numbers in the rows marked with ^{a,b,c} exponents are mutually statistically different (P < 0.05; P < 0.01 and P < 0.001).

Table 5**Effect of treatments on indicators of external and internal quality of eggs stored for 28 days at 4°C ($\bar{x} \pm s$)**

Indicators	A	B	C	P value
Egg length, mm	59.35±1.72	58.70±1.59	58.35±1.38	0.134
Egg width, mm	45.20±1.19	44.70±1.17	44.85±0.93	0.348
Shape index, %	76.17±1.33	76.18±2.00	76.88±1.36	0.281
Eggshell strength, kg/cm ²	3.39±0.68	3.39±0.58	0.366±0.59	0.299
Eggshell thickness, mm	0.427±0.01	0.424±0.02	0.421±0.02	0.732
Yolk color	12.45±0.82 ^a	12.00±0.72 ^{ab}	11.85±0.58 ^b	0.029
HU	70.04±9.39	72.32±7.15	75.18±5.21	0.101
Albumen height, mm	6.00±1.41	6.01±0.81	6.30±0.90	0.614
Albumen pH	9.14±0.05	9.16±0.06	9.17±0.05	0.393
Yolk pH	6.17±0.05	6.21±0.08	6.16±0.07	0.098

\bar{x} = arithmetic mean; s = standard deviation; The numbers in the rows marked with ^{a,b} exponents are mutually statistically different (P < 0.05)

between the two groups were not statistically significant ($p > 0.05$).

If we look at the values of HU shown in Tables 4 and 5 for eggs from experimental treatment measured in two periods, we notice that for fresh eggs higher values are in group C compared to groups B and A (80.21 HU in relation to 77.10 HU and 75.01 HU), and that their decreasing trend during storage of eggs is weaker in groups B and C compared to group A (6.22% and 6.27% compared to 6.62%). Such a trend for HU can be explained with reduced metabolic processes in the eggs of B and C groups, and it can be assumed that the cause is a higher content of selenium in the eggs of these groups and its antioxidant effect.

According to the specification for the device Egg multi tester which is used for measuring HU their values are classified into four grades of freshness. HU values above 72 represent eggs of best freshness labeled as AA, second is A grade, with HU values ranging from 71 to 60, followed by B grade

with values 59-31 and C grade with values of 30 and less. Average values of HU in all the groups were higher than the minimum required (72 HU) for fresh eggs of extra quality specified in the Egg multi tester EMT-5200.

Table 6 shows the results of the effects of treatment and time of analysis on the content of selenium in the edible part of the eggs. The results show that the treatment significantly affects the content of selenium in albumen, that is, with increasing levels of selenium in the diet the selenium content in albumen also increases ($p < 0.001$). Eggs that were analyzed on 14th day of the experiment had a significantly lower content of selenium in albumen compared to eggs analyzed on 26th day of the experiment ($p = 0.043$). Values of selenium content in egg yolks were in accordance with the analysis of the content of selenium in albumen. However, the treatment, time of the analysis and their interaction had no statistically significant effect ($p > 0.05$) on differences in the content of selenium in egg yolks. Surai and Sparks (2001), Gajčević et al. (2009)

Table 6
Effects of treatment and time of the analysis on the content of selenium in the egg (mg/kg sample)

Treatment	Time of analysis (days)	Number of samples	Albumen	Yolk
A	14	5	0.0783 ^c	0.5194
	26	5	0.0912 ^{bc}	0.5283
B	14	5	0.0877 ^c	0.5205
	26	5	1.0492 ^{ab}	0.5547
C	14	5	1.1117 ^a	0.5292
	26	5	1.1081 ^a	0.6001
SEM			0.005	0.023
Source of variation				
Treatment			<0.001	0.217
Time of analysis			0.043	0.054
Treatment x Time of analysis			0.114	0.412

T = treatment (A,B,C), TA = time of analysis (14th or 26th day of feeding); = standard error of the mean. The numbers in the columns marked with ^{ab,c} exponents are mutually statistically different ($P < 0.05$; $P < 0.01$ and $P < 0.001$)

and Gjorgovska et al. (2012) state that the level of selenium in feed has an impact on its content in albumen, which is consistent with the results of our research. These authors point out that the level of selenium also has a significant impact on the content of selenium in egg yolks, which was not found in our study.

Table 7 shows the shares (%) of basic parts of eggs kept in the refrigerator for 3 or 28 days at 4°C. The results show that the level of selenium in feed has no effect on the shares of egg albumens and yolks, while the influence of selenium was statistically significant for eggshell share ($C = 12.83\%$ and 12.70% $A > B = 12.19\%$; $p < 0.05$). For eggs stored for 28 days, it is evident that the level of selenium in feed has no statistically significant effect on the shares of the basic parts of the egg ($p > 0.05$).

Gjorgovska et al. (2012) state that the level of selenium in feed for laying hens has no impact on the share of the basic

Table
Shares of the basic parts of eggs (%; $\bar{x} \pm sd$)

Indicators	A	B	C	p value
3 days				
Share of albumen	61.45±1.07	61.79±1.83	61.86±1.67	0.675
Share of yolk	25.83±0.89	26.01±1.55	25.30±1.66	0.261
Share of shell	12.70±0.76 ^a	12.19±0.79 ^b	12.83±0.65 ^a	0.020
28 days				
Share of albumen	60.48±1.44	60.53±1.48	60.70±1.60	0.893
Share of yolk	26.85±1.12	26.69±1.22	26.59±1.60	0.818
Share of shell	12.65±0.62	12.76±0.79	12.70±0.58	0.882

\bar{x} = arithmetic mean; s = standard deviation; The numbers in the rows marked with ^{ab} exponents are mutually statistically different ($P < 0.05$)

parts of the egg ($p > 0.05$). They report that a group of hens that received the mixture with higher levels of selenium have eggs with a higher share of albumen ($G3 = 61.03\%$; $G2 = 60.47\%$ and $G1 = 59.33\%$), and lower shares of yolk ($G3 = 27.13\%$; $G2 = 27.18\%$ and $G1 = 28.61\%$) and shell ($G3 = 11.84\%$; $G2 = 12.35\%$ and $G1 = 12.06\%$) in comparison to the eggs of hens fed mixtures with lower levels of selenium. Their results are not consistent with ours. In this study we observed the freshness by measuring the intensity of lipid oxidation in egg yolks kept in a refrigerator at +4° C for 3 or 28 days.

Determination of the concentration of thiobarbituric acid reactive substances indicates the extent of oxidation of various fatty acids. The higher the concentration of TBARS, shown in $\mu\text{g MDA/g}$ sample, the greater is the extent of lipid oxidation. Figure 1 shows the intensity of lipid oxidation in egg yolks of experimental groups, measured in two periods. From the results it is evident that all values are equable, and that the treatment has no effect on lipid oxidation in egg yolks ($p > 0.05$). Gajčević et al. (2009) reported that levels of selenium have a significant impact on the intensity of lipid oxidation. The authors note that in a group of hens that were fed with higher levels of organic selenium TBARS value in egg yolks on 28th day was significantly lower compared to eggs originating from a group of hens that were fed with lower levels of organic selenium in the diet ($p < 0.05$). The results of those authors are not consistent with the results of this research. In addition to the production of eggs for consumption and reproduction, the importance of the addition of selenium-fortified grains in the feed stands out also in fattening of chickens, because it positively affects the quality of meat and enrichment of muscle tissue with selenium (Haug et al., 2008).

Conclusion

From the research results it can be concluded that the use of selenium-fortified wheat in hens' diets lead to greater supply of body with selenium, which is manifested in a higher

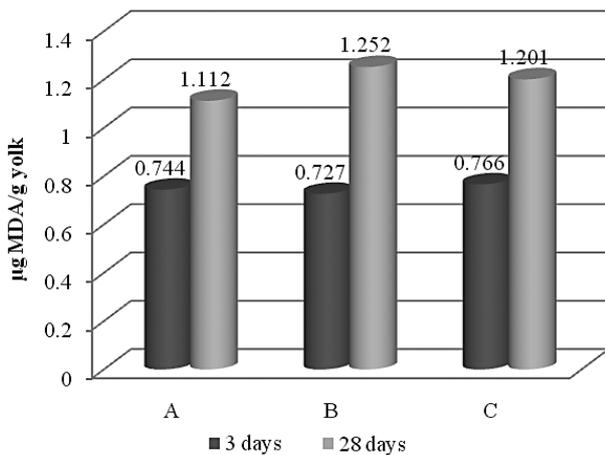


Fig. 1. The oxidation of lipids in egg yolks stored for 3 or 28 days in a refrigerator at +4°C

content of selenium in albumens of eggs from the experimental group C in regard to group A ($p < 0.001$), in both terms of measurements. The values of the selenium content in egg yolks also were higher in group C compared to groups B and A, but the differences were not statistically significant ($p > 0.05$). Furthermore, it was found that the level of selenium in feed has an impact on indicators of egg quality (weight of eggs and basic parts of eggs, length and width of the eggs, shell thickness and yolk color intensity) analyzed after 3 days of storage in the refrigerator. For eggs stored for 28 days in the refrigerator effect of treatment was recorded for the value of egg yolk color ($p = 0.029$). Values of lipid peroxidation measured in fresh eggs or eggs stored for 28 days in the refrigerator were equable in all groups, and treatment had no effect on this indicator of egg freshness ($p > 0.05$). In order to obtain even better results it is necessary to expand the research. Selenium-fortified corn and higher portion of selenium-fortified wheat should be added to hens' feed, and also, feeding experiment should last for a longer period of time (more than 26 days).

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