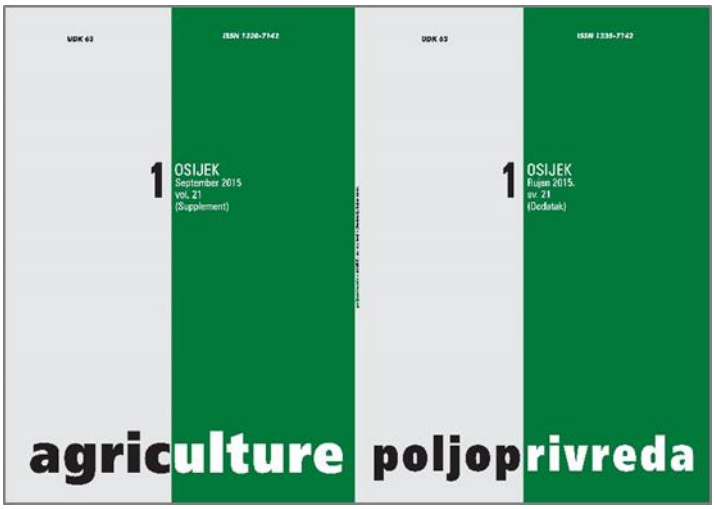
The effect of olive by products and their extracts on antioxidative status of laying hens and oxidative stability of eggs enriched with n-3 fatty acids

Rezar, V., Levart, A., Salobir, J.

Poljoprivreda/Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

http://dx.doi.org/10.18047/poljo.21.1.sup.51



Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek

Faculty of Agriculture in Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK: 636.52/.58+637.4:634.63
DOI: 10.18047/poljo.21.1.sup.51

THE EFFECT OF OLIVE BY PRODUCTS AND THEIR EXTRACTS ON ANTIOXIDATIVE STATUS OF LAYING HENS AND OXIDATIVE STABILITY OF EGGS ENRICHED WITH N-3 FATTY ACIDS

Rezar, V.(1), Levart, A.(1), Salobir, J.(1)

Original scientific paper

SUMMARY

The aim of the study was to assess the effects olive leaves, pulp and their extract supplementation on performance, antioxidant status and oxidative stability of eggs. Oxidative stress was induced by the addition of 6% linseed oil in the feed. 94 individually caged laying hens, 40 weeks old, were included in the study. Animals were divided into 6 groups. The feed of each group was composed of a basic feed, supplemented with: group Cont - no supplement, Vit E - 150 IU of α-tocopherol acetate /kg, Olive L - 1% of olive leaves, Olive Ex - extract from olive leaves, the Pulp group - 1% of dried and ground pulp and Pulp Ex - extract from pulp. Based on the results we found out that supplementation of vitamin E, olive leaves, pulp and their extracts had no effect on the performance of hens and showed neither a lymphocyte DNA damage preventive activity nor influence malondialdehyde (MDA) concentration in plasma. The results suggest that α-tocopherol acetate and olive leaves supplementation had significant effect on the MDA content of the stored eggs. Supplements, except vitamin E had neither influence on antioxidant activity (ACL) in eggs nor on n-3 PUFA in fresh and 40 days stored eggs.

Key-words: laying hens, nutrition, olive leaves, olive pulp, egg, oxidative stability

INTRODUCTION

Human dietary recommendations for general population have been focussed on increasing the consumption of polyunsaturated fatty acids (PUFAs), particularly from the n-3 series and reducing the consumption of saturated fatty acids (SFAs). It is also possible to increase the n-3 content of the yolk by enriching hen diets with flax oil. However, PUFAs are more prone to oxidation. These phenomena can be prevented or limited by enriching the eggs with antioxidants such as vitamin E. Feeding trials have shown that vitamin E is an efficient mean for improving the oxidative stability of eggs (Meluzzi et al., 2000). On the other hand natural feed additives in animal diets can also have antioxidant properties (Florou-Paneri et al., 2005). Olive leaves are agricultural residues from the beating of olive trees (*Olea europea L.*) for fruit harvest. They contain many substances, the most important are oleuropein, tyrosol and hydroxytyrosol (Silva et al., 2006). Most of the phenolic compounds are potent antioxidants with anti-inflammatory properties (Benavente–Garcia et al., 2000). Olive pulp is the raw material resulting from extraction of olive oil containing important phenolic compounds. The objective of the present study was to evaluate the effect of n-3 enriched feed supplemented with olive leaves, pulp or their extracts, on laying hen's performance, their antioxidant status and oxidative stability of eggs.

MATERIAL AND METHODS

The study included 94 Isa Brawn laying hens, 40 weeks old individually caged. The animals were randomly assigned to five treatment groups (Table 1). Hens within the control group (Cont) were fed a basal feed containing 6% linseed oil. Other five groups were fed the same diet supplemented with 150 IU/kg feed of α-tocopheryl acetate (Vit E), 10 g/kg of dried and ground olive leaves (Olive L), 1 g/kg of extract prepared from 10 g/kg of olive leaves (Olive Ex), 10 g/kg of dried and ground olive pulp (Pulp), and 1 g/kg of extract prepared from 10 g of olive pulp (Pulp Ex). The trial lasted for 6 weeks.

(1) Assist. Prof. Vida Rezar (vida.rezar@bf.uni-lj.si), Ph.D. Alenka Levart, Prof. Dr. Janez Salobir - University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

Table 1. Composition of the hen diets (per kg)

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex
Maize (g)	250.00	250.00	250.00	250.00	250.00	250.00
Wheat (g)	252.53	252.53	252.53	252.53	252.53	252.53
Sunflower meal (g)	80.00	80.00	80.00	80.00	80.00	80.00
Soybean meal (g)	207.82	207.82	207.82	207.82	207.82	207.82
Linseed oil (g)	60.00	60.00	60.00	60.00	60.00	60.00
Wheat starch (g)	10.00	10.00	/	9.00	/	9.00
Vitamin E (IU)	/	150	/	/	/	/
Olive leaves and olive leaves extract (g)	/	/	10.00	1.00	/	/
Olive pulp and olive pulp extract (g)	/	/	/	/	10.00	1.00

The mineral and vitamin calculated for the Isa Brawn hen's requirements

Fresh olive leaves from ecological olive trees and olive pulp were collected for extracts preparation in November. Leaves were dried at 50ºC and then ground to pass 2 mm screen and stored at 4ºC until used. Olive pulp was obtained from local oil mill, lyophilized and ground to pass 2 mm screen and stored as olive leaves. The 70% ethanolic extracts prepared from olive leaves and pulp including ethanol were evaporated. All diets were prepared each week in mash form and stored at 4ºC until use (Table 1).

Table 2. Chemical analysis of feed mixtures and n-3 fatty acids content (g/kg)

	Dry matter	Crude protein	Crude fat	Crude fibre	Crude ash	n-3 PUFA
Average ± std	911 ± 1.4	168 ± 2.2	78.6 ± 5.1	51.1 ± 2.2	144.7 ±7.4	42.2 ± 1.0

Live weight gain was recorded at the end of the experiment. Feed consumption was recorded weekly. The number of eggs and egg weights were recorded daily. At the end of the experiment, blood was collected for the determination of DNA fragmentation of blood lymphocytes, malondialdehyde (MDA) and ACL in plasma. The comet assay was performed by the method of Singh et al. (1988), with slight modifications as described by Rezar et al. (2003). The modified methodology of Wong et al. (1987) was used to measure the concentrations of MDA in blood plasma by HPLC. Plasma antioxidative capacity of lipid-soluble substances (ACL) in egg yolk was determined by the Photochem assay (Analytik Jena, Leipzig, Germany). Data were analysed using the GLM procedure of the SAS/STAT module (SAS 8e, 2000; SAS Institute Inc., Cary, NC). The study protocol was approved by the Animal Ethics Committee of the Veterinary Administration of the Republic of Slovenia.

RESULTS AND DISCUSSION

Poultry in intensive farming systems are frequently exposed to oxidative stress which can result in damage of the body proteins, lipids and DNA and can lead to reduce performance and health (Lykkesfeldt and Svendsen, 2007). The aim of the present study was to evaluate different natural feed additives (olive leaves and pulp) in preventing dietary-induced oxidative stress. Oxidative stress occurs due to inclusion of large amount of high-PUFA vegetable oils in animal feed for creating poultry products with improved nutritional quality (Wood et al., 2004). No differences between the groups were observed in body weight, feed consumption (Table 3), egg production and egg weight. The comparable data in literature are scarce. Botsoglou et al. (2005) did not find differences in egg weight and other egg quality characteristics in the experiment with different aromatic plants supplements. Also Jiang et al. (1994) reported no significant effect on egg weight, when α-tocopherol acetate was supplemented in hen diets at the level of 200 mg/kg.

Table 3. Results of hen's performance

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex	p
Body weight (g)	2105.7	2162.1	2062.6	2059.3	2013.7	2092.4	0.61
Feed intake (g/day)	109.4	113.5	108.8	110.8	104.0	114.8	0.07
Egg production (%)	98.4	96.2	95.2	96.2	93.7	98.0	0.09
Egg weight (g)	64.3	64.5	62.9	63.7	62.6	64.5	0.63

High concentrations of PUFA in the diet did not significantly increase the formation of MDA in plasma (Table 4). In contrast, Sahin et al. (2010) found out that the inclusion of resveratrol into diets could enhance antioxidant status in quails, as reflected by dose-dependent decreases in serum MDA. Oxidative damage to DNA is a useful index of oxidative stress. It was proposed that DNA damage is caused by free radical generated as a result of the high intake of PUFA. In our experiment no significant differences between the groups in the rate of lymphocyte DNA damage (head DNA percentage) were observed. Study of Fabiani et al. (2008) showed a potent DNA damage preventive activity of olive oil phenols in humans, but no such studies exist on laying hens.

Table 4. Lymphocytes DNA damage and the marker of oxidative stress MDA in blood plasma

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex	p
Plasma MDA (nmol/ml)	1.66	1.49	1.57	1.39	1.47	1.58	0.54
Head DNA (%)	79.47	76.41	79.59	80.58	76.44	80.56	0.69

The effect of dietary treatments on lipid oxidation of egg yolk, fresh or stored for 40 days is shown in Table 5. The obtained MDA values were very low and confirm generally high oxidative stability of fresh eggs (Cherian et al., 1996). The extend of lipid oxidation differed among the dietary treatments only in 40 days stored eggs and reveals lowest lipid oxidation in groups Vit E and Olive L. This is in agreement with Botsoglou et al. (2005) who found out that natural supplements (rosemary, oregano, saffron) improved oxidative stability of eggs in comparison to unsupplemented control group.

Table 5. MDA concentration (nmol/g egg yolk) in fresh and 40 days stored egg yolk

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex	P
Fresh	1.08	0.82	1.54	1.04	1.47	1.06	0.12
40 days stored	1.82^{a}	1.06^{b}	1.15^{b}	1.86^{a}	1.57^{ab}	1.42^{ab}	0.09

^{a,b,c,d} – Least squares means within a row without the same superscript differ significantly ($p<0.05$)

Antioxidative capacity of lipid-soluble substances (ACL) was measured in fresh and 40 days stored egg yolks (Table 6). In comparison with all other groups only vitamin E supplementation (group Vit E) significantly improved ACL in fresh and stored eggs.

Table 6. ACL (nmol/g egg yolk) in fresh and 40 days stored egg yolks

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex	p
Fresh	196.2^{a}	660.2^{b}	226.5^{a}	212.7^{a}	215.7^{a}	224.4^{a}	<0.0001
40 days stored	232.7^{a}	846.9^{b}	266.8^{a}	277.0^{a}	288.7^{a}	277.3^{a}	<0.0001

^{a,b,c,d} – Least squares means within a row without the same superscript differ significantly ($p<0.05$)

Table 7 shows that neither α-tocopherol acetate nor any other supplementation exerted any significant effect on the fatty acid composition of fresh eggs. Nevertheless, the results are in agreement with the previous studies reporting no effect of tocopherol supplementation on the fatty acids profile of fresh n-3 enriched eggs (Qi and Sim, 1998). Also, Botsoglou et al. (2012) reported that neither α-tocopherol acetate nor olive leaves supplementation exerted any change in fatty acids composition of eggs.

Table 7. Content of n-3 PUFA in fresh and 40 days stored egg yolk (% of total fatty acids)

	Cont	Vit E	Olive L	Olive Ex	Pulp	Pulp Ex	p
Fresh	12.6	13.8	13.5	14.2	13.8	14.3	0.58
40 days stored	12.6	13.5	13.2	14.2	13.6	14.3	0.39

CONCLUSION

Based on the results we can conclude that supplementation of vitamin E, olive leaves, pulp and their extracts had no effect on the hen performance. The parameters of oxidative stress (DNA damage and MDA concentration in plasma) showed that there was no effect of the supplements on the antioxidant status of the hens. Vitamin E exerted higher antioxidant activity (ACL) of eggs than other feed supplements used in the experiment. The results also show that the oxidative stability of lipids measured as MDA content in egg yolks was improved by vitamin E and olive leaves supplementation in 40 days stored eggs only, and did not change in fresh eggs. There was no effect of any supplement on the n-3 PUFA proportion in egg yolks.

REFERENCES

1. Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., Del Rijo, J.A. (2000): Antioxidant activity of phenolics from Olea europaea L. leaves. Food Chemistry, 68: 457–462. doi: http://dx.doi.org/10.1016/S0308-8146(99)00221-6
2. Botsoglou, N., Florou-Paneri, P., Botsoglou, E., Dotas, V., Giannenas, I., Koidis, A., Mitrakos, P. (2005): The effect of feeding rosemary, oregano, saffron and α-tocopheryl acetate on hen performance and oxidative stability of eggs. South African Journal of Animal Science, 35(3): 143-151. http://dx.doi.org/10.4314/sajas.v35i3.4053
3. Botsoglou, E., Govaris, A., Fletouris, D., Botsoglou, N. (2012): Effect of supplementation of the laying hen diet with olive leaves (Olea europea L.) on lipid eggs during storage. British Poultry Science, 53:4, 508-519. doi: http://dx.doi.org/10.1080/00071668.2012.720672
4. Cherian, G., Wolfe, F.W., Sim, J.S. (1996): Feeding dietary oils with tocopherols: Effects on internal qualities of eggs during storage. Journal of Food Science, 61: 15-18. doi: http://dx.doi.org/10.1111/j.1365-2621.1996.tb14716.x
5. Fabiani, R., Rosignoli, P., De Bartolomeo, A., Fuccelli, R., Servili, M., Montedoro, G., Morozzi, G. (2008): Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. The Journal of Nutrition, 138: 1411-1416.
6. Florou-Paneri, P., Nikolakakis, I., Giannenas, I., Koidis, A., Botsoglou, E., Dotas, V., Mitsopoulos, I. (2005): Hen performance and egg quality as affected by dietary oregano essential oil and a-tocopheryl acetate supplementation. International Journal of Poultry Science, 4: 449-454. doi: http://dx.doi.org/10.3923/ijps.2005.449.454
7. Jiang, Y., McGeachin, R., Bailey, C. (1994): α-tocopherol, ß-carotene, and retinal enrichment of chicken eggs. Poultry Science, 73: 1137-1143. doi: http://dx.doi.org/10.3382/ps.0731137
8. Lykkesfeldt, J., Svendsen, O. (2007): Oxidants and antioxidants in disease: Oxidative stress in farm animals. The Veterinary Journal, 173: 502-511. doi: http://dx.doi.org/10.1016/j.tvjl.2006.06.005
9. Meluzzi, J., Sirri, F., Manfreda G., Tallarico N., Franchini A. (2000): Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. Poultry Science, 79: 539-545. doi: http://dx.doi.org/10.1093/ps/79.4.539
10. Qi, G.H., Sim, J.S. (1998): Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. Journal of Agricultural and Food Chemistry, 46: 1920–1926. doi: http://dx.doi.org/abs/10.1021/jf9707804
11. Rezar, V., Pajk, T., Marinšek Logar, R., Ješe Janežič, V., Salobir, K., Orešnik, A. (2003): Wheat bran and oat bran effectively reduce oxidative stress induced by high-fat diets in pig. Annals of Nutrition and Metabolism, 47: 78-84. doi: http://dx.doi.org/10.1159/000069279
12. Sahin, K., Akdemir, F., Orhan, C., Tuzcu, M., Hayirli, A., Sahin, N. (2010): Effects of dietary resveratrol supplementation on egg production and antioxidant status. Poultry Science, 89: 1190-1198. doi: http://dx.doi.org/10.3382/ps.2010-00635
13. Silva, S., Gomes, L., Leitao, F., Coelho, A.V., Vilas Boas, L. (2006): Phenolic compounds and antioxidant activity of Olea europaea L. fruits and leaves. Food Science and Technology International, 12: 385-395. doi: http://dx.doi.org/10.1177/1082013206070166
14. Singh, N. P., McCoy, M.T., Tice, R.R., Schneider, E.L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175: 184-191. doi: http://dx.doi.org/10.1016/0014-4827(88)90265-0
15. Wong, S.H., Knight, J.A., Hopfer, S.M., Zaharia, O., Leach, C.N., Sunderman, F.W. (1987): Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clinical Chemistry, 33: 214-220.
16. Wood, J.D., Richardson, R.I., Nute, G.R, Fisher, A.V., Campo, M.M., Kasapidou, E., Sheared, P.R., Enser, M. (2004): Effect of fatty acids on meat quality: A review. Meat Science, 66: 21-32. doi: http://dx.doi.org/10.1016/S0309-1740(03)00022-6

(Received on 3 June 2015; accepted on 20 July 2015)