EFFECTS OF DIETARY SUPPLEMAENTATION BY HYDROPHILIC EXTRACT FROM EDIBLE MASHROOM (*Flammulina velutipes*) TO LAYING HENS ON OXIDATIVE STABILITY OF HEN EGGS

Jeong-Ho Sohn, Yusuke Sagara and Toshiaki Ohshima

Department of Food Science and Technology, Graduate School of Tokyo University of Marine Science and Technology, Konan 4, Minato-ku, Tokyo, Japan

Abstract – The present study was conducted to investigate the effects of dietary supplementation with the hydrophilic extract prepared from mushroom (*Flammulina velutipes*) trimming waste on hen egg quality in terms of lipid oxidation of egg yolk. 2-Mercaptohistidine trimethylbetaine (L-ergothioneine, EGT) contents of the egg yolk and white laid by hens fed on diets added with the extract were higher than those laid by the hens fed diets without the extract. The EGT contents of the egg yolk and white increased with the prolongation in the feeding period of the extract and reached to 9-fold and 14-fold, respectively of the basal levels of EGT after 26 days of dietary supplementation. The residual oxygen in the glass vials, in which the lyophilized powder of the yolks laid by the supplemented hens were sealed, was significantly ($P<0.05$) lower compared to those in the control group. Phospholipids in the yolk of the supplemented group were significantly ($P<0.05$) higher compared to those of the control group. In conclusion, dietary supplementation of the mushroom extract in the diet of laying hens was accomplished not only to enhance the EGT contents of the egg yolk and white, but also to increase the stability against lipid oxidation of the yolk.

Key Words – antioxidant, ergothioneine, hens, supplementation.

I. INTRODUCTION

Hen egg contains valuable nutrients such as protein, lipids, vitamins and minerals and is one of the most important food sources to maintain human health. There have been a number of reports on the effects of dietary supplementation on egg quality such as nutritional benefit and functional properties. Dietary supplementation of fermented Ginkgo-leaves in layer diets may be a feasible means of producing eggs of lower cholesterol and higher PUFA contents (1). Fatty acid composition and carotenoid content of egg yolk are improved by the addition of marine hemicroalgae in laying hen diet (2). Moreover, dietary supplementation with wild ginseng adventitious root meal increases egg production, reduces serum cholesterol concentrations in laying hen (3).

2-Mercaptohistidine trimethylbetain (L-ergo-thioneine, EGT) is an amino acid analogue that was first discovered from ergot fungus of a devastated rye grain (4). EGT is biosynthesized from L-histidine, cysteine, and methionine, with hercynine and hercynylcysteine sulfoxide concerned as an intermediate. EGT is biosynthesized exclusively in certain fungi and some bacteria, but not in any animals or higher plant species. There have been a lot of studies on the physiological function of EGT, including inhibition of discoloration and lipid peroxidation (5,6), attenuation of postprandial triglyceride concentrations (7), protection against UV and gamma irradiation (8), prevention or alleviation of disease and anti-inflammatory (9,10), and prevention of melanosis (11,12). Mushroom contains EGT ranging from 0.4 to 2.0 mg/g dry weight and thus is one of the best dietary source to obtain EGT, and (13).

The aim of the present study was to evaluate the effects of supplementing diets with extract mushroom processing waste including EGT on laying hen. Furthermore, the abilities of EGT as an antioxidant to prevent the lipid oxidation of egg yolk power were investigated.

II. MATERIALS AND METHODS

A total of 10 laying hens (*Gallus gallus* domesticus) of 28 weeks age were selected based on their body weights. The hens were distributed individually into cages and provided with artificial light during 14 h/day. They were given free access

to a basal diet and water before the start of dietary supplementation. The feeding plan was designed to use a 120 g of control diet without mushroom extract and a supplementation diets added with 12 g of the mushroom extract to the control diet per a hen. The supplementation diet was fed to all of laying hens from 1st till 5th weeks, and then the supplementation diet was replaced to the control diet which was fed to hens from 6th to 10th weeks. This feeding cycle was repeated; the supplementation diet and the control diet were fed to hen from 11th till 15th and 16th till 20th weeks, respectively.

For measurement of oxygen absorption to evaluate lipid oxidation, a 3 g portion of the lyophilized egg yolk powder was taken in a glass vials (50 ml in vol), and the vial was sealed with a Teflon-lined septum. For hydroperoxide analyses, a 3 g portion of the lyophilized egg yolk was taken in polyethylene bags and stored in an incubator controlled at 50 °C for 15 days.

Contents of EGT in the egg yolk and white were quantitatively measured by high-performance liquid chromatography (HPLC) with a post column reaction system between EGT and 2,2´-dipyridyl disulphidewith slight modification (15).

The residual oxygen percentages of the yolk lyophilized powder in the vials were quantified by gas chromatography.

Phospholipids in the yolk lyophilized powder were quantitatively determined by normal phase HPLC using a silica column.

Microsoft Excel 2007 was used to calculate means and standard deviation (SD). One-way analysis of variance was used to distinguish significant differences among the mean values. A statistically significant difference between two mean values was declared at $P<0.05$.

III. RESULTS AND DISCUSSION

The EGT contents in the egg yolk and white of the hens fed on diets with the mushroom extract increased markedly with prolongation of the dietary supplement period, and the EGT contents elevated to around 9-fold and 14-fold of the basal levels, respectively after 26 days of dietary supplementation. Once the supplementation of the extract was suspended, the EGT contents of both of the egg yolk and white turned to decline rapidly and reached to the initial levels to those before starting the dietary supplementation. By adding the extract to the hen's diet again after 71 days, the EGT contents in egg yolk and the white turned to increase again. By the second suspension in supplementation of the extract after 105 days, the EGT contents in the egg yolk and white decreased again (Figs. 1 and 2).

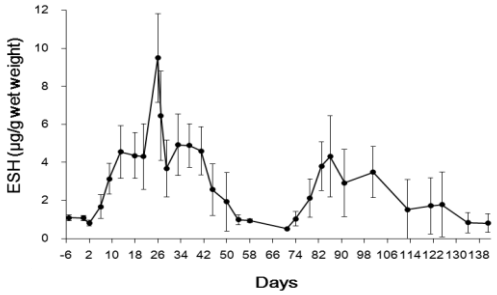

Figure 1. Changes in ergothioneine contents of the egg yolk during dietary supplementation of mushroom hydrophilic extract.

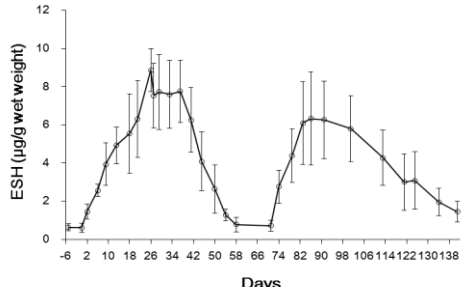

Figure 2. Changes in ergothioneine contents of the egg white during dietary supplementation of mushroom hydrophilic extract.

The percentages of residual oxygen in the head space of the vials in which the egg yolk powder were sealed decreased gradually during the storage period as shown in Fig. 3. Here the residual oxygen of the treatment group was significantly ($P<0.05$) higher than that of the control group after 9 days of storage.

The total phospholipids of both control and supplemented groups decreased slightly throughout storage period as shown in Fig. 4. The area ratio of phospholipids in the treatment group was significantly ($P<0.05$) higher than that of the control group after 15 days of storage.

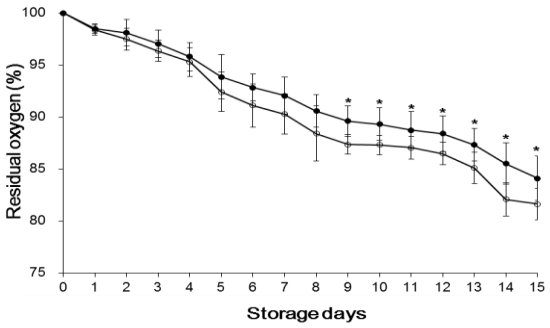

Figure 3. Changes in residual oxygen of the control and treatment egg yolk powders during storage at 50 °C.

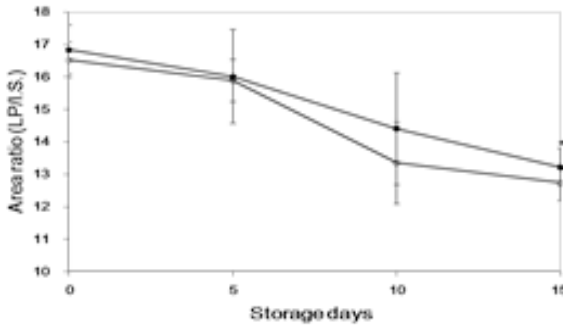

Figure 4. Changes in phospholipids of the control and treatment egg yolk powders during storage at 50 °C.

This study showed that the EGT contents in both of the egg yolk and white obtained from the hens supplemented the mushroom extract increased remarkably with prolongation of the dietary supplementation period. EGT at a level of 6.5 mg/day was provided to a laying hen throughout 120 g of diet added with mushroom processing waste extract in the present study. The higher EGT contents in the egg yolk and white obtained in the present study suggested that supplementation of the mushroom extract to hens resulted in the development of EGT-enriched eggs. These results were coincident with a previous study in which the levels of EGT in the blood of pig which were fed on grain became higher although pig fed on purified casein was not detected of the EGT in blood. The level of EGT in the muscle of Kuruma shrimp (*Marsupenaeus japonicas*) were also elevated by dietary supplementation of the mushroom extract (15), the whole blood of yellowtail fish (*Seriola quinqueradiata*) and the muscle of cattle (16). On the other hand, Ey et al. (17) reported that the EGT contents in commercial egg yolk and white were 0.68 and 0.38 µg/g wet weight, respectively. Concentration of EGT in the red blood cell of male human reaches a maximum level at the age of 18, and then it decreases slightly (18). Moreover, the rate of EGT accumulation in the body is different depending on the type of cell and tissues. The accumulations of EGT in the body tissue are associated with degree of cells with expression of EGT transport (SLC22A4) (19). A high level of EGT in the tissues is intimately related with the high levels of EGT transport. Also, they have functionally validated EGT transport from chicken, along with many other species such as mouse, pig and zebrafish. Consequently, in the present study, the higher EGT contents in the egg yolk and white suggest that the egg perhaps contained enough EGT transport to accumulate EGT from hen tissues.

The oxidation of unsaturated fatty acid is one of the most fundamental reactions in lipid chemistry. In the present study, the decrease of oxygen in the both glass vials indicated that oxygen was used for autoxidation of lipid in yolk powder. However, lipids in the control group seemed more unstable compared to the treatment group after 9 days of storage. The treatment group has higher residual oxygen percentage than control group, it might be due to the antioxidant activity present in EGT of dietary supplement containing the mushroom extract.

We could conclude that the oxidative decomposition of egg yolk powder was suppressed by dietary supplementation of the mushroom extract. This suppression of lipid oxidation will be due to ergothioneine in the extract.

IV. CONCLUSION

Dietary supplementation of the mushroom extract to laying hens promoted accumulation of EGT in egg yolk and white, moreover the stronger antioxidative effects of EGT significantly suppressed the residual oxygen and PL decomposition in the egg yolk powder during storage. Decline of hen eggs quality in terms of the oxidative stability by handling, processing and

preservation will be improved by supplementation of mushroom EGT to layer hens.

ACKNOWLEDGEMENTS

Feeding trials to hens were carried out in Brown-Egg Farm Co., Ltd. in Nagano Prefecture, Japan.

REFERENCES

1. Zhao, L., Zhang, X., Cao, F., Sun, D., Wang, T., Wang, G., 2013. Effect of dietary supplementation with fermented Ginkgo-leaves on performance, egg quality, lipid metabolism and egg-yolk fatty acids composition in laying hens. Livestock Science. 155, 77-85.
2. Fredriksson, S., Elwinger, K., Pickova, J., 2006. Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chemistry. 99, 530–537.
3. Yan, L., Meng, Q.W., Ao, X., Wang, J.P., Jang, H.D., Kim, I.H., 2011. Evaluation of dietary wild-ginseng adventitious root meal on egg production, egg quality, hematological profiles and egg yolk fatty acid composition in laying hens. Livestock Science. 140, 201-205.
4. Tanret, C., 1909. Sur une base nouvelle retiree du seigle ergote, L-ergothioneine, Compt. Rend. 149, 222–224.
5. Bao, H.N.D., Shinomiya, Y., Ikeda, H., Ohshima, T., 2009. Preventing discoloration and lipid oxidation in dark muscle of yellowtail by feeding an extract prepared from mushroom (Flammulina velutipes) cultured medium. Aquaculture. 295, 243–249.
6. Bao, H.N.D., Ochiai, Y., Ohshima, T., 2010. Antioxidative activities of hydrophilic extracts prepared from the fruiting body and spent culture medium of Flammulina velutipes. Bioresource Technology. 101, 6248–6255.
7. Weigand-Heller, A.J., Kris-Etherton, P.M., Beelman, R.B., 2012. The bioavailability of ergothioneine from mushrooms (Agaricus bisporus) and the acute effects on antioxidant capacity and biomarkers of inflammation. Preventive Medicine. 54, S75–S78.
8. Hartman, P.E., Hartman, Z., Citardi, M.J., 1988. Ergothioneine, histidine, and two naturally occurring histidine dipeptides as radioprotectors against gamma-irradiation inactivation of bacteriophages T4 and P22, Radiat. Res. 114, 319–330.
9. Deiana, M., Rosa, A., Casu, V., Piga, R., Dessi, M.A., Aruoma, O.I., 2004. L-Ergothioneine modulates oxidative damage in the kidney and liver of rats in vivo: studies upon the profile of polyunsaturated fatty acids. Clinical Nutrition. 23, 183–193.
10. Repine, J.E., Elkins, N.D., 2012. Effect of ergothioneine on acute lung injury and inflammation in cytokine insufflated rats. Preventive Medicine. 54, S79–S82.
11. Encarnacion, A.B., Fagutao, F., Shozen, K., Hirono, I., Ohshima, T., 2011. Biochemical intervention of ergothioneine-rich edible mushroom (Flammulina velutipes) extract inhibits melanosis in crab (Chionoecetes japonicus). Food Chemistry. 127, 1594–1599.
12. Encarnacion, A.B., Fagutao, F., Jintasataporn, O., Worawattanamateekul, W., Hirono, I., Ohshima, T., 2012. Application of ergothioneine-rich extract from an edible mushroom Flammulina velutipes for melanosis prevention in shrimp, Penaeus monodon and Litopenaeus vannamei. Food Research International. 45, 232–237.
13. Dubost, N.J., Beelman, R., Peterson, D., Royce, D.J., 2006. Identification and quantification of ergothioneine in cultivated mushrooms by liquid chromatography-mass spectroscopy. International Journal of Medicinal Mushrooms. 8, 215–222.
14. Nguyen, T.H., Giri, A., Ohshima, T., 2012. A rapid HPLC post-column reaction analysis for the quantification of ergothioneine in edible mushrooms and in animals fed a diet supplemented with extracts from the processing waste of cultivated mushrooms. Food Chemistry. 133, 585–591.
15. Encarnacion, A. B., Fagutao, F., Hirono, I., Ushio, H., Ohshima, T., 2010. Effect of ergothioneine from mushrooms (Flammulina velutipes) on melanosis and lipid oxidation of kuruma shrimp (Marsupenaeus japonicus). Journal of Agricultural and Food Chemistry. 58, 2577–2585.
16. Bao, H.N.D., Ushio, H., Ohshima, T., 2008. Antioxidative activity and antidiscoloration efficacy of ergothioneine in mushroom (Flammulina velutipes) extract added to beef and fish meats. Journal of Agricultural and Food Chemistry. 56, 10032–10040.
17. Ey, J., Schömig, E., Taubert, D., 2007. Dietary Sources and Antioxidant Effects of Ergothioneine. J. Agric. Food Chem. 55, 6466-6474.
18. Kumosani, T.A., 2001. L-ergothioneine level in red blood cells of healthy human males in the Western province of Saudi Arabia, Exp. Mol. Med. 33, 20–22.
19. Gründemann, D., 2012. The ergothioneine transporter controls and indicates ergothioneine activity-A review. Preventive Medicine. 54, S71–S74.
20. Cheah, I.K., Halliwell, B., 2012. Ergothioneine; antioxidant potential, physiological function and role in disease. Biochim. Biophys. Acta. 1882, 784–793.