ENHANCEMENT OF ANTIOXIDATIVE PROPERTIES OF HEN TISSUES BY DIETARY SUPPLEMENTATION OF HYDROPHILIC EXTRACTS FROM EDIBLE MUSHROOMS

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Abstract – The authors have reported that dietary supplementation with the hydrophilic extract prepared from mushroom (*Flammulina velutipes*) trimming waste accomplished not only to enhance the contents of 2-mercaptohistidine trimethylbetaine (L-ergothioneine, EGT) in the egg yolk and white, but also to increase the stability against lipid oxidation of the yolk. Based on the previous results, the present study was conducted to investigate effects of dietary supplementation with the similar hydrophilic extract of mushroom trimming wastes on the antioxidative properties of laying hens in terms of free radical scavenging ability of blood and internal organs. A significant amount of EGT accumulated in egg, breast meat, and internal organs by supplementation. The largest EGT uptake was observed in liver, followed by kidney, heart, gizzard, and breast meat in this order. No negative effects on the egg weights were found in feeding the diet containing the mushroom extracts. The mushroom EGT was incorporated from the supplemented diet into the blood of the laying hens and resulted in suppressing the accumulation of lipid hydroperoxides. DPPH radical scavenging ability was also observed associated with the accumulation of EGT in the blood. Vertical migration from the supplemented hens to chicks was recognized in the bloods, livers, hearts, and yolks. In these organs DPPH radical scavenging ability were also enhanced by incorporated EGT.

Key Words – antioxidative property, ergothioneine, hens, radical scavenging ability.

I. INTRODUCTION

There have been a number of reports on the effects of dietary supplementation to laying hens 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 microalgae 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). The authors have reported that supplementation of edible mushroom hydrophilic extract enhanced the incorporation of 2-mercaptohistidine trimethylbetain (L-ergo-thioneine, EGT) in egg yolk and white.

Certain fungi and bacteria biosynthesize EGT exclusively, but not in any animals or higher plant species. It is well accepted that EGT prohibits discoloration and lipid peroxidation (4,5), attenuation of postprandial triglyceride concentrations (6), protection against UV and gamma irradiation (7), prevention or alleviation of disease and anti-inflammatory (8,9), and prevention of melanosis (10,11).

The aim of the present study was to evaluate the effects of supplementing diets with the extract of mushroom processing waste including EGT on antioxidative properties of laying hens and chicks.

II. MATERIALS AND METHODS

A total of 40 laying hens (*Gallus gallus* domesticus) of 28 weeks age were selected based on their body weights and distributed individually into cages and provided with artificial light during 14 h/day. Four experimental groups with 10 hens each were set in the feeding trial. Individual hen was given free access to a basal diet (BD) and water for 10 days prior to the start of dietary supplementation. The feeding plan was designed to use a 100 g of supplementation diet added with four different amounts of the mushroom extract to the BD per a hen as shown in Table 1.

Table 1. Compositions of four experimental diets

1. Ferrule solid 11 g (EGT 4.4 mg) ＋BD100 g
2. Ferrule solid 5.5 g (EGT 2.2 mg) ＋BD 100 g
3. Concentrated hot water extract 3.2 mL (EGT 4.4 mg) ＋BD 100 g
4. Concentrated hot water extract 1.6 mL (EGT 2.2 mg) ＋BD 100 g

*Basal diet

The supplementation diet was fed to all of laying hens for 5 weeks. Chicks (10 females and 12 males) hatched from the eggs, which EGT-supplemented parent birds laid, were kept under fasting condition with free taking the water for 4 days. Then the chicks were dissect for further analyses. The animal experiments were carried out according to the enforcement rule of the university.

Contents of EGT in blood and internal organs were quantitatively analyzed by high-performance liquid chromatography (HPLC) with a post column reaction system between EGT and 2,2'-dipyridyl disulphide with slight modification (12).

DPPH radical scavenging activity was quantitatively measured by HPLC with a post column reaction system, according to Nguyen et al. (13).

Microsoft Excel 2007 was used to calculate means and standard deviation. 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

Incorporation of EGT into muscle and internal organs

As already found in the previous study, EGT accumulated in eggs. The amounts of EGT in the breast meat and certain internal organs increased by supplementation.

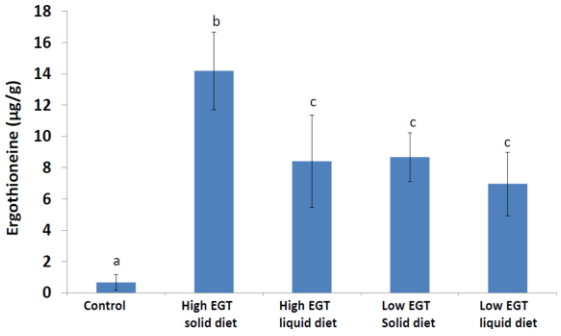

Fig. 1. Ergothioneine contents in hens breast meats supplemented by mushroom extract.

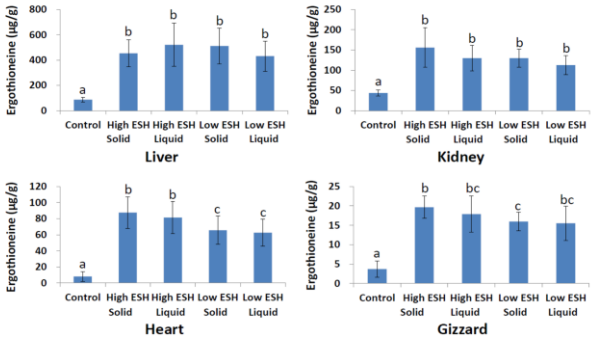

Fig. 2. Ergothioneine contents in the internal organs of hens supplemented by mushroom extract.

The largest uptake was recognized in the liver, followed in order by kidney, heart, gizzard, and breast meat as shown remarkably in Figs. 1 and 2.

Typical chromatograms in Fig. 3 represent clearly that not only EGT but also DPPH radical scavenging ability (RSA) increased in the blood by supplemental feeding of the mushroom extract. Positive correlations between the amounts of EGT

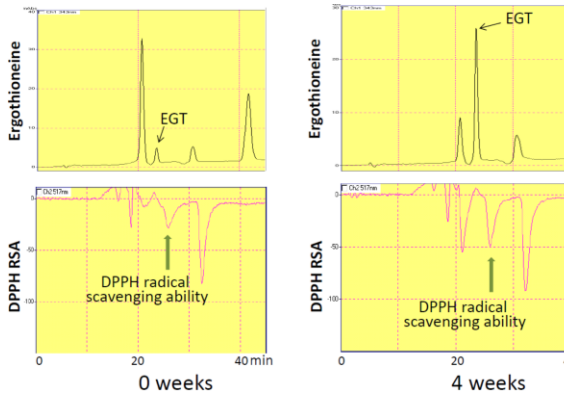

Fig. 3. Typical HPLC chromatograms of ergothioneine and its related DPPH radical scavenging ability of hens blood.

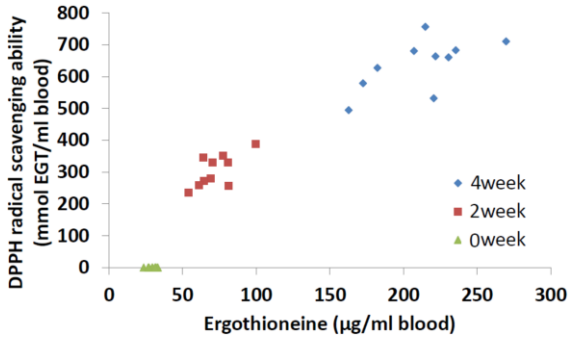

Fig. 4. Relationship between ergothioneine contents and radical scavenging ability of hens blood.

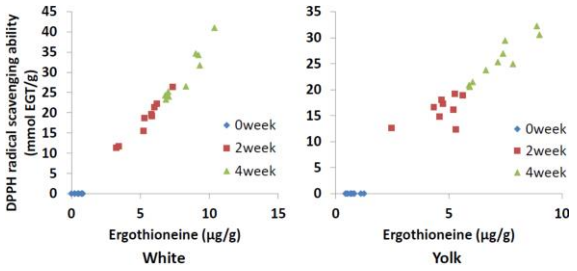

Fig. 5. Relationship between ergothioneine contents and radical scavenging ability of hens eggs supplemented by the mushroom extract.

and intensity of DPPH RSA in the hens' blood (Fig.4) as well as in the egg yolks and whites (Fig.5) were recognized.

Inherit of ergothioneine from parent lying hens to chicks

The chicks hatched from the eggs with a large amount of incorporated EGT had remarkable amounts of EGT in the blood as well as the tissues of certain internal organs even efter the animals were kept under fasting for 4 days. These specimens rich in EGT showed higher DPPH RSA as shown in Fig. 6.

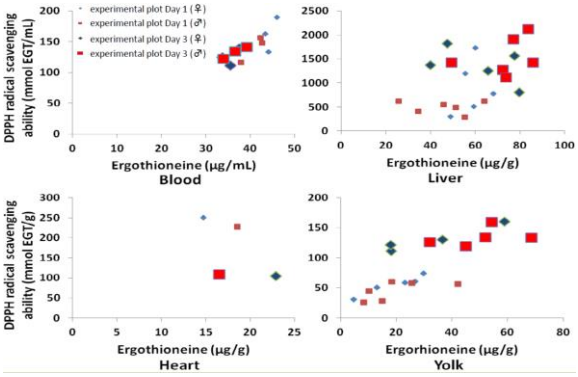

Fig.6. Relationship between ergothioneine contents and radical scavenging ability of the blood and several internal organs of newborn chicks hatched from the eggs enriched in ergothioneine.

It is well accepted that EGT is biosynthesized from L-histidine, cysteine, and methionine with hercynine and hercynylcysteine sulfoxide concerned as an intermediate in certain fungi and bacteria. Animals or higher plant species, however, can't biosynthesize EGT exclusively (14). The present study clearly showed that the certain tissues and blood specimens contained lower level of EGT even though prior to feeding the mushroom extract. It will be reasonable therefore to consider the EGT existed in these specimens was incorporated from the diets which the animals were usually fed. Contrary to this, the animals fed on the extract incorporated a considerable amount of EGT. The detailed mechanism to explain these results is still unclear, however it is probably due to organic cation/carnitine transporter 1 (OCTN1) which is also recognized to play as a transporter of EGT in humans and certain vertebrates such as mice, rats and cattle. Indeed, EGT disappeared in the OCTN1 knockout mouse, suggesting that OCTN1 is not biosynthesized in mouse (15).

IV. CONCLUSIONS

The facts obtained in the present study, EGT inherited from the parent hens to chicks enhanced radical scavenging abilities in several tissues, suggest EGT plays some important role for chicks, such as protecting newborn chicks from oxidative stress. Further investigation on the physiological significance of EGT for chicks will be required.

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