Short Communication

The Antioxidant Status of Serum and Egg Yolk in Layer Fed with Mushroom Stembase (Flammulina velutipes)

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A B S T R A C T

The objective of this study was to evaluate the antioxidant properties of serum and egg yolk in ISA Brown layer hens fed with mushroom (Flammulina velutipes) stembase (FVS). A total 150 hens of 30 wk old were grouped into 5 equal treatments with 5 replications of 6 hens each. Dietary treatments included basal diet as a control group; control diet including antibiotic (0.05% flavomycin) as an antibiotic group; 2%FVS fed group; 4% FVS fed group and 6% FVS fed group. The experimental duration was total 63 days, from 30 wk to 39 wk. Serum total antioxidant (T-AOC) was significantly higher (P<0.05) in 4%FVS than the control group and antibiotic fed groups; MDA was significantly lower (P<0.05) in FVS fed groups and antibiotic groups than in the control fed group. However, there was no significant difference were observed for serum total superoxide dismutase (T-SOD) in among experimental groups. Yolk antioxidant T-AOC and T-SOD were significantly higher (P<0.05) in 4%FVS than the control group and antibiotic fed groups; MDA was significantly lower (P<0.05) in FVS fed groups than antibiotic group and control fed groups. Mushroom stembase can be used as a dietary supplement at 6%level to improve the antioxidant capacity of serum and egg yolk in ISA Brown laying hens.

Antioxidants are a group of compounds that inhibit oxidation and reduce free radicals directly or indirectly in body system (Liu et al., 2014). Oxidation and generation of free radicals are part of the normal body metabolites in the host organisms. In any environmental stress, these highly reactive chemicals can be overproduced and may cause abnormalities and dysfunction of the body system (Liu et al., 2014). Excessive free radicals can affect animal performance and even lead to the development of diseases (Moncol, 2007). In addition, Mujahid (2007) and Zhao (2011) reported that the antioxidant status can improve production performance in birds. Oxidative stress in the body can be relieved by exogenous suplemental antioxidants (Rahman, 2007). However, several synthetic antioxidants have shown potential side effects such as liver damage and carcinogenesis, especially long-term use of synthetic antioxidants (Rautou, 2010). Therefore, exploring safe and natural antioxidants to defend against oxidative stress has become a body of research in recent years.

The conventional use of butylated hydroxyanisole and butylated hydroxytoluene as synthetic antioxidants may have a public human hazard, so it is necessary to discover natural antioxidant products (Ser et al., 2016).

Different herbs have been used as chemotherapeutics in the poultry industry (Kamran et al., 2016). Besides, Metin et al. (2017) reported that natural plants and agro-industrial bye products can be used as potential ingredients in poultry industry.

Flammulina is a kind of edible mushroom that can provide key nutrients such as protein, vitamins, minerals, unsaturated fatty acids and fiber (Reis, 2012; Mahfuz et al., 2017). In addition, F. velutipes mushroom is a good source of protein (Kim, 2009). The main amino acids in F. velutipes are methionine, valine, isoleucine, leucine, lysine, phenylalanine and threonine, L-glutamic acid, L-alanine, glycine and L-lysine (Beluhan and Xia, 2015). Therefore, the polysaccharides of F. velutipes have good antioxidant properties. The various proportion of F. velutipes mushroom have been reported as a potential source of antioxidants (Tang et al., 2016).

As the production of F. velutipes is increasing year
Materials and methods

Experimental chickens (ISA Brown) were purchased from Changchun Octavia farms and *F. velutipes* stembase (FVS) was collected from the local domestic mushroom farm in Changchun city, Jilin, China.

A single factor completely randomized design was used to select 150 healthy, 210-day-old ISA brown laying hens and were randomly divided into 5 groups. Each group had 5 replicates and each had 6 hens. The control group was fed the basal diet, the antibiotic group was fed with the basal diet with the addition of antibiotic, (flavomycin, 0.05%) and the 2%FVS, 4%FVS and 6%FVS fed groups were added with 2%, 4%, and 6% of the ground mushroom stembase on the basal diet. The experimental period is 63 days. The basal dietary nutrition level of laying hens is based on the nutritional requirements of laying hens in the NRC (1994). Feeds and water were provided *ad libitum* in the whole trial period. The hen house temperature was 24°C and a 17 h light with 7 h dark period was maintained throughout the whole experimental period.

The mushroom stembase sample was prepared (0.01mm) by laboratory high speed universal sample grinder (Huanghua xinxing electric Appliance Co, Hebei, China). Six representative samples in triplicate were obtained and analyzed for proximate components dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), total minerals (Ash) including calcium and phosphorus following the method of AOAC. Nitrogen was determined using an FP-528 nitrogen determinator (LECO Corporation, USA). Total phenolics content was measured according to the method of Giannenas *et al.* (2010). The value was expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g) on dry weight basis. The analyzed nutritional composition of the experimental diets and FVS were presented in Table I and Table II, respectively.

At the end of the experiment at d 273 (39 wk), blood samples were obtained via wing vein from 5 hens per experimental group. Serum was obtained by centrifuged at 3000 × g for 20 min at 4°C (Legend Micro 17R centrifuge, Thermo Fisher, Germany) and were stored at -80°C until measuring serum antioxidant properties. Similarly, at the end of the experimental period at d 273 (39 wk), fresh eggs were selected (5 eggs per treatment) and egg yolks were collected for the test. Malondialdehyde (MDA) content, total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, and total antioxidant capacity (T-AOC) in serum and egg yolk were measured using commercial kits provided by Nanjing Jiancheng Bioengineering Institute, Nanjing, China (MDA A003-1, T-SOD A001-1, GSH-Px A005, T-AOC A015-3).

### Table I

| Ingredients            | Control | Anti-biotic | 2% FVS | 4% FVS | 6% FVS |
|------------------------|---------|-------------|--------|--------|--------|
| Maize corn             | 577.0   | 576.5       | 562.0  | 545.0  | 533.0  |
| Soyabea meal           | 263.0   | 263.0       | 259.0  | 258.0  | 254.0  |
| Soyabea oil            | 31.0    | 31.0        | 30.0   | 28.0   | 24.0   |
| FVW<sup>a</sup>        | -       |             |        |        |        |
| Lysine                 | 1.0     | 1.0         | 1.0    | 1.0    | 1.0    |
| Methionine             | 2.0     | 2.0         | 2.0    | 2.0    | 2.0    |
| Dicalcium              | 30.0    | 30.0        | 30.0   | 30.0   | 30.0   |
| Limestone              | 92.0    | 92.0        | 92.0   | 92.0   | 92.0   |
| Common salt            | 2.0     | 2.0         | 2.0    | 2.0    | 2.0    |
| Vit-mineral premix<sup>b</sup> | 2.0       | 2.0         | 2.0    | 2.0    | 2.0    |
| Antibiotics            | -       | 0.5         | -      | -      | -      |
| Total                  | 1000    | 1000        | 1000   | 1000   | 1000   |

**Chemical analysis**

| Material | Control | Anti-biotic | 2% FVS | 4% FVS | 6% FVS |
|----------|---------|-------------|--------|--------|--------|
| DM (g/kg) | 912.4       | 921.2       | 923.3  | 923.5  | 924.10 |
| CP (g/kg) | 168.3       | 168.0       | 169.1  | 168.8  | 169.3  |
| Ca (g/kg) | 40.6        | 40.40       | 41.11  | 41.0   | 40.6   |
| P (g/kg)  | 7.10        | 7.05        | 7.15   | 7.20   | 7.20   |
| EE (g/kg) | 51.4        | 52.1        | 52.10  | 51.30  | 51.60  |
| CF (g/kg) | 25.6        | 25.5        | 29.60  | 33.7   | 37.90  |

**Calculated analysis**

| Material | Control | Anti-biotic | 2% FVS | 4% FVS | 6% FVS |
|----------|---------|-------------|--------|--------|--------|
| ME (MJ/kg) | 11.55       | 11.50       | 11.61  | 11.59  | 11.64  |
| Lysine (g/kg) | 10.4       | 10.4        | 10.30  | 10.5   | 10.4   |
| Methionine (g/kg) | 5.0         | 4.9         | 5.0    | 4.8    | 4.9    |
| Cystine (g/kg)   | 2.6        | 2.6         | 2.7    | 2.5    | 2.6    |

<sup>a</sup>FVS= *F. velutipes* stembase at 2%, 4% and 6%.<sup>b</sup>Provided per kg of the complete diet: retinyl acetate, 4500 IU; cholecalciferol, 1200 IU; DL-a-tocopheryl acetate, 25000 IU; thiamin, 5000 mg; riboflavin, 20000 mg; phylloquinone,10000 mg; niacin, 45000 mg; pantothentic acid, 35000 mg; biotin, 1500 mg; folic acid, 1000 mg; cyanocobalamin, 40 mg; zinc, 45 mg; manganese 50 mg; iron, 30 mg; copper, 4 mg; cobalt, 120 µg; iodine, 1 mg; selenium, 120 µg. <sup>2</sup>DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus; EE, crude fat; CF, crude fiber; ME, metabolisable energy.

One-way analysis of variance (ANOVA) was performed using SPSS 15.0. The results were expressed as the mean plus SEM value (standard error), and each set of data was processed using Duncan’s multiple comparison method.

Results and discussion

Serum antioxidant T-AOC was significantly
higher (P<0.05) in 6%FVS than control; GSH-Px was significantly higher (P<0.05) in all levels of mushroom fed groups and control groups than antibiotic fed groups; MDA was significantly lower (P<0.05) in all levels of mushroom fed groups and antibiotic groups than control fed groups. However, there was no significant difference were observed for T-SOD among experimental groups (Table III).

Table II.- Chemical compositions and active ingredients of *F. velutipes* mushroom stem base (FVS).1

| Chemical composition and active ingredients | Value                  |
|--------------------------------------------|------------------------|
| Moisture (g/kg)                            | 116.0 ±0.85            |
| Crude protein (CP, %Nx6.25) (g/kg)         | 137.5±0.75             |
| Crude fiber (CF) (g/kg)                    | 22.05±0.11             |
| Ether extract (EE) (g/kg)                  | 28.0±0.01              |
| Ash (total minerals) (g/kg)                | 116.1±0.09             |
| Organic matter (OM) (g/kg)                 | 884.0±0.85             |
| Nitrogen free extract (NFE) (g/kg)         | 498.0±3.45             |
| Calcium (ca) (g/kg)                        | 1.8±0.14               |
| Available phosphorus (p) (g/kg)            | 6.4±0.28               |
| Total phenolic content (mg, GAE/g)         | 6.87±0.25              |

1Values are expressed as the mean ± standard deviation (n=6).

For yolk antioxidant T-AOC, and T-SOD were significantly higher (P<0.05) in 4%FVS than the control group and antibiotic fed groups; MDA was significantly lower (P<0.05) in all levels of mushroom fed groups than antibiotic group and control fed groups (Table IV).

This study assumed that mushroom contains different bioactive components especially phenolic compound, the amino acids that may role on improving the antioxidant capacity. The phenolics compounds, has the antioxidants activities, was discovered from the mushroom *F. velutipes* (Rahman *et al*., 2015). The various proportion of *F. velutipes* mushroom has been reported as a potential source of antioxidants (Tang *et al*., 2016). Form some past studies it has been known that the polysaccharides and oligosaccharide present in *F. velutipes* mushroom shows the antioxidant function (Ma *et al*., 2015; Xia, 2015). Liu *et al*. (2016) reported that different polysaccharides originated from *F. velutipes* mushroom residue were purified and the antioxidant function has been considered in the study. Zeng *et al*. (2012) stated that *F. velutipes* mushroom holds the higher phenolic with the highest antioxidant activities. Besides, *F. velutipes* mushroom was found to exhibit vitamin-C that may play a role in antioxidant function (Tang *et al*., 2016). In a recent study by Han *et al*. (2017) found that dietary inclusion of yeast selenium and selenite could improve the activity of glutathione peroxidase in serum, and improve the activity of superoxide dismutase in the liver in chicken. Adding polyphenols in feed could increase the activity of glutathione peroxidase and superoxide dismutase in serum and improve the total antioxidant capacity of serum in weaned pigs (Chen *et al*., 2018). In addition, adding L- arginine in the diet of laying hens could improve the total antioxidant capacity and reduce the concentration of malondialdehyde in egg yolk and serum (Duan *et al*., 2015).

Tables III.- Effects of mushroom stembase on serum antioxidant in layer.

| Parameters | Control | Antibiotic | 2%FVS | 4%FVS | 6%FVS | SEM  | P value |
|------------|---------|------------|-------|-------|-------|------|---------|
| T-AOC      | 0.71±e  | 1.14<sup>bc</sup> | 1.45<sup>bc</sup> | 1.33<sup>b</sup> | 1.95<sup>e</sup> | 0.124 | 0.01    |
| MDA        | 19.71<sup>c</sup> | 10.53<sup>a</sup> | 15.36<sup>b</sup> | 15.6<sup>b</sup> | 13.62<sup>a</sup> | 0.847 | 0.03    |
| T-SOD      | 64.23<sup>a</sup> | 67.03<sup>a</sup> | 74.47<sup>a</sup> | 70.97<sup>a</sup> | 75.17<sup>a</sup> | 1.924 | 0.56    |
| GSH-Px     | 878.68<sup>b</sup> | 683.6<sup>a</sup> | 767.21<sup>b</sup> | 865.57<sup>b</sup> | 832.78<sup>b</sup> | 22.984 | 0.01    |

T-AOC, total antioxidant capacity; MDA, malondialdehyde; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase. 1data represent the mean value of 5 samples for each treatment (n=5). 2<sup>bc</sup> means in the same row with different letters are significantly different at P<0.05. SEM, pooled standard error of the means.

Tables IV.- Effects mushroom stembase on egg yolk antioxidant in layer.

| Parameters | Control | Antibiotic | 2%FVS | 4%FVS | 6%FVS | SEM  | P value |
|------------|---------|------------|-------|-------|-------|------|---------|
| T-AOC      | 1.16<sup>a</sup> | 1.15<sup>a</sup> | 1.17<sup>a</sup> | 1.74<sup>b</sup> | 1.44<sup>a</sup> | 0.074 | 0.02    |
| MDA        | 18.11<sup>b</sup> | 18.16<sup>b</sup> | 14.83<sup>a</sup> | 13.09<sup>a</sup> | 13.71<sup>a</sup> | 0.611 | 0.01    |
| T-SOD      | 41.74<sup>a</sup> | 42.79<sup>a</sup> | 51.98<sup>b</sup> | 58.11<sup>b</sup> | 47.69<sup>a</sup> | 2.386 | 0.01    |

T-AOC, total antioxidant capacity; MDA, malondialdehyde; T-SOD, total superoxide dismutase. 1data represent the mean value of 5 samples for each treatment (n=5). 2<sup>a</sup> means in the same row with different letters are significantly different at P<0.05. SEM, pooled standard error of the means.
Conclusion

The present study explored that FVS could be a good source of natural feed supplement as well as antioxidant that can improve both serum and yolk antioxidant properties. Focus on quality eggs production and sound health status, *F. velutipes* mushroom stem base can be used as a natural supplement as well as a substitute for antibiotic on layer production.

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Statement of conflicts of interest

Authors have declared no conflict of interest.

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