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Short Communication

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

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Authors' Contribution
MC conducted this experiment work under the supervision of ZL and HS. YC and LJ helped in mushroom preparation, experimental diet and participated in the animal experiment. MC, YC and LJ worked with lab test. SM, MC and LJ helped in statistical analysis and formatting the manuscript.

Key words
Feed supplement, laying hens, mushroom stembase, serum antioxidant, yolk antioxidant

ABSTRACT

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 6%FVS than control; glutathione peroxidase (GSH-Px) was significantly higher ($P<0.05$) in FVS fed groups and control groups than in the antibiotic fed group; Malondialdehyde (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 supplemental 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 Ranogajec, 2011). Yang (2015), Ma *et al*. (2015) and Xia *et al*. (2015) have reported that the polysaccharides in *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

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by year, that leads to the availability of the stem waste material. This sty focuses on the utilization of mushroom stembase materials. Here the effect of feeding mushroom stembase on antioxidant status of serum and egg yolk in laying hens, has been evaluated.

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^{0}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^{o}C (Legend Micro 17R centrifuge, Thermo Fisher, Germany) and were stored at -80^{o}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.- The ingredients and chemical composition of experimental diets (g/kg).

Ingredients	Control	Anti-biotic	2% FVS	4% FVS	6% FVS
Maize corn	577.0	576.5	562.0	545.0	533.0
Soyabean meal	263.0	263.0	259.0	258.0	254.0
Soyabean oil	31.0	31.0	30.0	28.0	24.0
FVW[a]	-	-	20.0	40.0	60.0
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[b]	2.0	2.0	2.0	2.0	2.0
Antibiotics	-	0.5	-	-	-
Total	1000	1000	1000	1000	1000
Chemical analysis[c]					
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					
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

[a] FVS= *F. velutipes* stembase at 2%, 4% and 6%. [b]Provided per kg of the complete diet: retinyl acetate, 4500 IU; cholecalciferol, 1200 IU; DL-α-tocopheryl acetate, 25000 IU; thiamin, 5000 mg; riboflavin, 20000 mg; phylloquinone,10000 mg; niacin, 45000 mg; pantothenic acid, 35000 mg; biotin, 1500 mg; folic acid, 3000 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. [c]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

[1]Values 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^{a}	1.14^{bc}	1.45^{bc}	1.33^{b}	1.95^{c}	0.124	0.01
MDA	19.71^{c}	10.53^{a}	15.36^{b}	15.6^{b}	13.62^{b}	0.847	0.03
T-SOD	64.23	67.03	74.47	70.97	75.17	1.924	0.56
GSH-Px	878.68^{b}	683.6^{a}	767.21^{ab}	865.57^{b}	832.78^{b}	22.984	0.01

T-AOC, total antioxidant capacity; MDA, malondialdehyde; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase. [1]data represent the mean value of 5 samples for each treatment (n=5). [a, b,c] 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^{a}	1.15^{a}	1.17^{a}	1.74^{b}	1.44^{a}	0.074	0.02
MDA	18.11^{b}	18.16^{b}	14.83^{a}	13.09^{a}	13.71^{a}	0.611	0.01
T-SOD	41.74^{a}	42.79^{ab}	51.98^{ab}	58.11^{b}	47.69^{ab}	2.386	0.01

T-AOC, total antioxidant capacity; MDA, malondialdehyde; T-SOD, total superoxide dismutase. [1]data represent the mean value of 5 samples for each treatment (n=5). [a, b,]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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