Mushroom (*Flammulina velutipes*) stem residue on growth performance, meat quality, antioxidant status and lipid metabolism of broilers

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**ABSTRACT**

Mushroom (*Flammulina velutipes*) stem residue (MSR) is rich with bioactive ingredients. This study aimed to assess the role of mushroom stem residue (MSR) on growth performance, meat quality, antioxidant status, and lipid metabolism of broilers. A total 168 Arbour Acres male broilers were assigned into 3 treatments (7 replications, 8 chicks each) and fed a basal diet as control (CON); 1% mushroom stem residue containing diet (1% MSR); and 2% MSR containing diet. Superoxide dismutase, glutathione peroxidase, and catalase value in serum, thigh muscle, breast muscle and liver sample, were higher (linear and quadratic, \( p < .05 \)) in MSR groups than that of CON. Malondialdehyde content in serum, thigh muscle, breast muscle and liver tissue sample, were lower (linear and quadratic, \( p < .05 \)) in MSR diets than CON. Compared with control, total cholesterol value of liver sample was lower (linear and quadratic, \( p < .05 \)) in 2% MSR diet on day 21 and day 42. Triglyceride value was also lower (linear and quadratic, \( p < .05 \)) in both levels of MSR diets than control on day 21 and day 42. MSR at 2% level can be applied on improving antioxidant status and lipid metabolism of broilers.

**HIGHLIGHTS**

- *Flammulina velutipes* mushroom stem residue (MSR) contains different bio-active ingredients which can be used in broiler diets.
- MSR had no any adverse effects on growth performance and normal meat quality of broilers.
- MSR could improve different antioxidant enzyme activities in breast muscle, thigh muscle, liver tissue and serum of broilers.
- MSR could reduce cholesterol levels in liver tissue of broilers.
- MSR at 2% level can be applied on improving antioxidant status and lipid metabolism in broilers.

**Introduction**

Mushrooms are rich with nutritional components as well as pharmacological properties (Adams et al. 2019). Medicinal mushrooms and their by-products in broilers as dietary supplementation as well as for alternative to antibiotics have achieved the priority since last few years (Mahfuz et al. 2020). In addition, mushrooms contain different active components which have been proved to be applied as production enhancing feed supplement in livestock (Bonanno et al. 2019). *Flammulina velutipes* mushroom possesses an excellent source of protein with all types of essential amino acids, vitamins, minerals, fibres as well as unsaturated fatty acids (Tang et al. 2016). *Flammulina velutipes* is enriched with antioxidant (Ma et al. 2015; Xia 2015; Liu et al. 2016; Guo et al. 2019), immune-modulatory (Li et al. 2011; Wu et al. 2014; Tang et al. 2016; Chuang et al. 2020), and cholesterol lowering functions (Park et al. 2007; Wu et al. 2010; Yeh et al. 2014; Chuang et al. 2020). Higher market demand of edible mushroom *Flammulina velutipes*, may lead greater yield of mushroom stem residue and is treated as waste materials. Currently, more attempts have paid to utilise the agricultural by-products as animal feed, as well as to minimise the cost of production and reducing environmental pollution (Shi et al. 2012; Al-Harthi et al. 2019; Biondi et al. 2020). Mushroom stem residue is the lower base part of mushroom stem and it is obtained after harvesting the fruiting.
body of mushroom. Approximately 100,000 tons of stem residue per year was retained from *Flammulina velutipes* mushroom industry in China and it was supposed that the yield is also greater in other countries of the world (Japan, South Korea, America and Europe) (Guo et al. 2019). Till now, a small part of *Flammulina velutipes* stem base is treated as compost materials where the major remaining portions are wasteful (Liu et al. 2016).

In recent years, consumers are paying more attention to organic poultry products. Dietary supplementation can be an effective way to improve meat quality in broiler production). On the other hand, the issue regarding the antibiotic resistance leads an increased force to reduce the in-feed antibiotics in poultry (Shang et al. 2016). Thus, animal scientists are searching for antibiotics alternative from herbal resources to produce organic poultry product. Similarly, poultry researchers as well as feed industry is trying to establish antibiotics free feeds from unconventional natural feed supplement to produce organic products from chickens (Anderson 2009; Mahfuz and Piao 2019).

Few studies have been done before with mushroom stem residue on growth performance, meat quality, antioxidant status and lipid profile in broilers. The current experiment hypothesised that dietary mushroom stem residue (MSR) will influence on antioxidant enzyme activities, and improve lipid metabolism in broilers. Taking into consideration the aim of this research was to examine the role of MSR as a natural supplement on growth performance, meat quality, antioxidant status and lipid metabolism in broilers.

**Materials and methods**

**Research design, birds and diets**

The research was conducted at chicken experimental unit, China Agricultural University, China and approved by Animal Care and Use Committee of China Agricultural University (Beijing, China, CAU-XSPLAB-B-2019-03). A total 168 Arbour Acres (AA) male broilers (1-day old, initial body weight 45.13 ± 1.04g) were randomly assigned into 3 treatments (7 replications of 8 chicks for each treatment) including a basal diet as control (CON); 1% mushroom stem residue (1% MSR); and 2% MSR diets respectively. Adequate feed and fresh water were supplied at the entire treatment period of 42 days.

Two different types of diets (starter diet for 0–21 days; finisher diet for 22–42 days) were considered to meet the nutrient levels followed by National Research Council (NRC, 1994) specification. All broilers were kept in an environment-controlled room and housed in a three-layer wire cages (120 cm–60 cm–50 cm). Initial brooding house temperature was 34 °C and it was decreased by 3 °C for per week until reaching at 23 °C. The relative initial humidity (RH) of the chicken house was 50% and increased by 5% per week up to 70%. Lighting duration was 24 h at beginning 3 days and then maintained 23 h constant till the end of trail.

**Chemical analyses of diets and mushroom stem residue**

All diet samples including mushroom stem residue (MSR) sample were prepared (0.01 mm) for analysis of dry matter, crude protein, crude fibre, ether extract, total minerals, calcium and phosphorus according to AOAC (2004). The amount of amino acids was estimated by specific analyser (Hitachi L-8800, Tokyo, Japan). Gross energy (MJ/kg) in diets was determined by a calorimeter (Parr 6400, Moline, IL). The major active component β-glucan content was determined following a previous method (Sari et al. 2017) and phenolics amount (mg GAE/g) was estimated as described previously (Fu et al. 2002). The nutritional levels of diets and analysed components of mushroom stem residue are shown in Tables 1 and 2, respectively.

**Growth performance and relative weight of inner organs**

Feed consumption and body weight gain were recorded on the days 21 and 42. After that, feed conversion ratio (FCR) was calculated.

The birds were selected to be bled from jugular vein and euthanized (cervical dislocation) at the end of trial (day 42; n = 7). Inner organs including heart, liver, kidney, pancreas, lung, thymus, bursa, spleen, and intestine were removed from all euthanized birds and weighed. Individual birds were weighed before scarified and the relative organs weight were presented (% live weight).

**Sample collection and analysis for antioxidant and lipid profile**

At sample collecting days (days 21 and 42), blood samples were obtained from jugular vein (n = 7). Serum was prepared by centrifuging at 3000 × g for 20 min at 4 °C and was preserved at −80 °C for further analysis. Antioxidant parameters including total
antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA) in serum, thigh muscle, breast muscle and liver tissue sample were determined using commercial assay kits (Beijing Kangjia Bioengineering Company, Beijing, China). The thigh muscle sample (left portion), breast muscle sample (left portion), and liver sample (left liver lobe) were collected, washed (0.9% ice-cold saline), and made small pieces following the method described before (Shang et al. 2014). Briefly, a homogenate solution was made with phosphate buffer (pH 7.4), which was then centrifuged at 12,000 \( g \) for 10 min at 4 °C. The supernatant fluid was used to detect the antioxidant enzyme activity, malondialdehyde (MDA) content, and lipid profile activities in tissue samples. The concentrations of total cholesterol (TC), and triglyceride (TG) in liver sample were measured following the manufacturer instructions of corresponding reagent kits (Zhongsheng Biochemical Co. Ltd., Beijing, China) via an automatic biochemical analyser (RA-1000, Bayer Corp, Tarrytown, NY). The breast muscle (left, pectoralis major) sample and the thigh muscle (left, biceps femoris) sample were sealed separately plastic bag and preserved at 4 °C for further analysis of meat quality.

### Meat quality analysis

After slaughtering, the breast and thigh muscle pH value were measured at 45 min (pH45min) and 24 h (pH24h) at 3 locations via portable pH metre (pH – star, Matthias, Germany). Duplicate measurement of sample was done and the average values were considered as the final values. The determination method of pH was followed by Shang et al. (2014). The drip loss of breast muscle and thigh muscle were measured according to Cheng et al. (2019). Briefly, the samples were trimmed and weighed, then placed in an inflated plastic bag and hung for 24 h at 4 °C. After 24 h, the samples were weighed again. Drip loss was calculated as a percentage based on weight before and after hanging. The colour value of breast muscle and thigh

### Table 1. Ingredients and nutrient composition of the experimental diets (% as fed)

| Items                  | Starter (1-21day) | Finisher (22-42day) |
|------------------------|-------------------|---------------------|
|                        | CON 1%MSR 2%MSR   | CON 1%MSR 2% MSR    |
| Corn                   | 59.46 58.56 57.66 | 65.04 64.25 62.72   |
| Soybean meal           | 28.83 28.73 27.53 | 23.18 23.07 22.70   |
| Soybean oil            | 3.0 3.0 3.0       | 3.40 3.40 4.0       |
| Corn gluten meal       | 2.50 2.50 3.0    | 3.0 3.0 3.0         |
| Fish meal              | 2.50 2.50 2.50    | 2.0 2.0 2.0         |
| MSR1                   | 1.0 1.0 2.0      | 1.0 1.0 2.0         |
| Di-calcium phosphate2  | 1.50 1.50 1.50    | 1.04 1.04 1.04      |
| Limestone              | 1.25 1.25 1.25    | 1.34 1.34 1.34      |
| Premix3                | 0.50 0.50 0.50    | 0.50 0.50 0.50      |
| Salt                   | 0.30 0.30 0.30    | 0.30 0.20 0.20      |
| Methionine2 (98%)      | 0.13 0.13 0.13    | 0.08 0.08 0.08      |
| Lysine-L2 (98%)        | 0.02 0.02 0.02    | 0.09 0.09 0.09      |
| Threonine2 (98%)      | 0.01 0.01 0.01    | 0.03 0.03 0.03      |
| Total                  | 100 100 100       | 100 100 100         |
| Chemical analysis (%)  |                  |                     |
| Dry matter             | 88.05 87.77 88.01 | 87.96 87.46 87.68   |
| Crude protein          | 21.01 21.0 21.03  | 19.02 19.03 19.02   |
| Ether extract          | 5.65 5.66 5.93    | 6.13 6.13 6.70      |
| Crude fiber            | 2.81 3.01 3.17    | 2.56 2.76 2.95      |
| Calcium                | 1.01 1.2 1.01     | 0.90 0.91 0.91      |
| Phosphorus             | 0.46 0.44 0.44    | 0.35 0.34 0.35      |
| Calculated analysis    |                  |                     |
| Metabolizable energy (MJ kg\(^{-1}\)) | 12.7 12.7 12.7 | 13.1 13.1 13.1 |
| Lysine (%)             | 1.10 1.10 1.10    | 1.0 0.99 1.0        |
| Threonine (%)          | 0.80 0.80 0.80    | 0.73 0.73 0.73      |
| Methionine (%)         | 0.50 0.49 0.49    | 0.38 0.39 0.40      |
| Cystine (%)            | 0.32 0.31 0.32    | 0.28 0.28 0.29      |
| Tryptophan (%)         | 0.28 0.28 0.28    | 0.24 0.24 0.24      |

MSR=Flammulina velutipes mushroom stem residue.

1Commercial available source.

2Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 10,050 IU; vitamin D3, 2,800 IU; vitamin E (DL-a-tocopheryl acetate), 50 mg; vitamin K3, 3.5 mg; thiamine, 2.5 mg; riboflavin, 7.5 mg; pantothenic acid, 15.3 mg; pyridoxine, 4.3 mg; vitamin B12(cyanocobalamin), 0.02 mg; niacin, 35 mg; choline chloride, 1,000 mg; biotin, 0.20 mg; folic acid, 1.2 mg; Mn, 100 mg; Fe, 85 mg; Zn, 60 mg; Cu, 9.6 mg; I, 0.30 mg; Co, 0.20 mg; and Se, 0.20 mg.

CON: control
**Table 2.** Chemical compositions of *F. velutipes* mushroom stem residue (% DM)*.

| Items                        | Value  |
|------------------------------|--------|
| Proximate components (%)     |        |
| Dry matter                   | 84.26  |
| Crude protein (CP, N × 6.25) | 13.33  |
| Crude fibre (CF)             | 21.62  |
| Ether extract (EE)           | 1.73   |
| Ash (total minerals)         | 8.72   |
| Organic matter (OM)          | 91.30  |
| Nitrogen free extract (NFE)  | 50.78  |
| Calcium                      | 0.38   |
| Phosphorus                   | 0.88   |
| Energy (MJ/kg)               | 15.35  |
| Amino acid components (g/kg) |        |
| Aspartic acid                | 10.5   |
| Threonine                    | 5.3    |
| Serine                       | 5.0    |
| Glutamic acid                | 20.8   |
| Proline                      | 5.6    |
| Glycine                      | 4.9    |
| Alanine                      | 7.4    |
| Valine                       | 5.5    |
| Isoleucine                   | 4.9    |
| Leucine                      | 7.3    |
| Tyrosine                     | 5.3    |
| Phenylalanine                | 5.7    |
| Histidine                    | 2.0    |
| Lysine                       | 7.8    |
| Arginine                     | 4.8    |
| Cystine                      | 1.4    |
| Methionine                   | 1.8    |
| Tryptophan                   | 1.5    |
| Active ingredients: (β-glucan (g kg⁻¹)) | 1.85 |
| Total phenolic content (mg, GAE g⁻¹) | 6.88 |

*Values are expressed as the mean value (n = 3). MSR: *Flammulina velutipes* mushroom stem residue; DM: dry matter.

**Muscle** were estimated in duplicates using a Chromameter (CR-410, Konica Minolta, Tokyo, Japan). The test units L*, a*, and b* indicated the lightness, redness and the yellowness value of meat respectively.

**Statistical analysis**

One-way analysis of variance (ANOVA) was applied via SPSS (2006). Individual broiler was the experimental unit for trial. Polynomial contrasts were applied for linear and quadratic value for the levels of dietary mushroom stem residue. Tukey’s multiple range test was applied to separate the statistical differences. Mean value and SEM were used to express results. Statements of significant level was as p < .05.

**Results**

**Analysis of mushroom stem residue**

The analysed nutritional composition and major active ingredients of mushroom stem residue (MSR) are shown in Table 2. The data showed that dry matter (DM), crude protein (CP) and crude fibre (CF) content in MSR were at satisfactory level as 84, 13 and 21% respectively. Gross energy of MSR was 15.4 MJ kg⁻¹.

The types of essential amino acid of MSR were presented (Table 2). The major active ingredients, i.e. the total phenolic content (mg, GAE g⁻¹) was 6.88; and β-glucan was 1.85 (g kg⁻¹).

**Growth performance and inner organs weight**

No significant differences (p > .05) was observed for the feed consumption, average daily body weight gain, feed conversion ratio and final body weight among the groups (Table 3).

The bursa weight was higher (linear, p < .05) in broilers offered MSR compared with control (CON), whereas no significant differences (p > .05) were noted for other inner relative organs weight (heart weight, liver weight, kidney weight, pancreas weight, lung weight, intestinal weight and lymph node weight) in the groups.
weight, thymus weight, spleen weight and intestine weight) (Table 4).

**Meat quality traits**

No significant \((p > .05)\) effects were noted for pH value, drip loss, and the colour value (lightness; redness; yellowness) in both breast and thigh muscle among the groups (Table 5).

**Serum antioxidant status**

On day 21, superoxide dismutase (SOD) value and glutathione peroxidase (GSH-PX) value were higher (linear and quadratic, \(p < .05\)) in broilers offered with MSR at both levels compared with control (CON) (Table 6). Catalase enzyme activity (CAT) value was also higher (linear and quadratic, \(p < .05\)) in 2% MSR diet compared with CON. The value for malondialdehyde (MDA), was lower (linear and quadratic, \(p < .05\)) in broilers offered with MSR diets compared with CON.

On day 42, SOD value and GSH-PX value were higher (linear and quadratic, \(p < .05\)) in 2% MSR group than CON. CAT value was also higher (linear and quadratic, \(p < .05\)) in broilers offered MSR diets than CON. MDA value was lower (linear and quadratic, \(p < .05\)) in broilers offered MSR diets than CON. Total antioxidant capacity (T-AOC) value was not affected by mushroom stem residue supplementation in this study, but the value was greater \((p > .05)\) in 2% MSR compared with CON at the both evaluating periods.

**Muscle tissue antioxidant status**

Antioxidant status in muscle tissue (thigh and breast muscle) of experimental broilers is presented in Table 7. Superoxide dismutase (SOD) value, glutathione peroxidase (GSH-PX) value, catalase enzyme activity (CAT) value of thigh muscle and breast muscle were significantly higher (linear and quadratic, \(p < .05\)) in broilers

### Table 5. Effect of *F. velutipes* mushroom stem residue (MSR) on meat quality in broilers on day 42\(^a\).

| Items              | CON | 1%MSR | 2%MSR | SEM  | T    | L    | Q    |
|--------------------|-----|-------|-------|------|------|------|------|
| Breast muscle      |     |       |       |      |      |      |      |
| pH-45 min          | 6.21| 6.00  | 6.04  | 0.06 | .536 | .190 | .387 |
| pH-24 hour         | 5.43| 5.43  | 5.44  | 0.02 | .182 | .683 | .451 |
| Drip loss (%)      | 3.14| 2.95  | 3.09  | 0.19 | .80  | .960 | .624 |
| Color\(^b\)        |     |       |       |      |      |      |      |
| L                  | 49.9| 48.1  | 50.3  | 0.49 | .452 | .921 | .574 |
| a\(^*\)            | 14.85| 15.22| 14.66| 0.39 | .948 | .974 | .989 |
| b\(^*\)            | 13.42| 12.58| 14.87| 0.63 | .564 | .453 | .358 |
| Thigh muscle       |     |       |       |      |      |      |      |
| pH-45 min          | 6.11| 6.06  | 6.01  | 0.04 | .649 | .315 | .515 |
| pH-24 hour         | 5.91| 5.59  | 5.73  | 0.05 | .168 | .145 | .089 |
| Drip loss (%)      | 2.39| 2.36  | 2.36  | 0.20 | .385 | .697 | .538 |
| Color\(^b\)        |     |       |       |      |      |      |      |
| L                  | 53.91| 54.74| 52.87| 0.76 | .879 | .724 | .763 |
| a\(^*\)            | 18.43| 19.29| 18.98| 0.544| .601 | .465 | .718 |
| b\(^*\)            | 14.09| 16.90| 13.70| 0.571| .198 | .885 | .214 |

\(^{a}\)Data represented the mean value of 7 samples per treatment. CON: control; MSR: *Flammulina velutipes* mushroom stem residue; SEM: pooled standard error of the means; T: overall effect of treatment; L, linear effect of increasing MSR; Q, quadratic effect of increasing MSR (1% and 2% diet). a,b, – means in the same row with different letters are significantly different at \(p < .05\).

\(^{b}\)L\(^*\), lightness; a\(^*\), redness; b\(^*\), yellowness.

### Table 6. Effect of *F. velutipes* mushroom stem residue (MSR) on serum antioxidant status in broilers\(^1\).

| Items              | CON | 1%MSR | 2%MSR | SEM  | T    | L    | Q    |
|--------------------|-----|-------|-------|------|------|------|------|
| Day 21             |     |       |       |      |      |      |      |
| SOD (U/mL)         | 2.38| 2.38  | 2.48  | 0.03 | .498 | .277 | .313 |
| GSH-PX (U/mL)      | 41.05| 50.61| 51.13| 1.60 | .023 | .008 | .032 |
| CAT (U/mL)         | 18.56| 23.29| 25.76| 1.09 | .001 | .001 | .001 |
| MDA (nmol/ml)      | 1.59| 1.25  | 1.21  | 0.06 | .001 | .001 | .001 |
| Day 42             |     |       |       |      |      |      |      |
| SOD (U/mL)         | 1.39| 1.40  | 1.45  | 0.02 | .677 | .304 | .475 |
| GSH-PX (U/mL)      | 42.74| 47.33| 53.72| 1.87 | .006 | .003 | .002 |
| CAT (U/mL)         | 6.67| 11.61| 12.27| 0.78 | .021 | .010 | .021 |
| MDA (nmol/ml)      | 1.33| 1.22  | 1.16  | 0.10 | .035 | .035 | .019 |

\(^1\)Data represented the mean value of 7 samples per treatment. CON: control; MSR: *Flammulina velutipes* mushroom stem residue; T-AOC: total antioxidant capacity; SOD: superoxide dismutase; GSH-PX: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde. SEM: pooled standard error of the means; T, overall effect of treatment; L, linear effect of increasing MSR; Q, quadratic effect of increasing MSR (1% and 2% diet), a,b, – means in the same row with different letters are significantly different at \(p < .05\).

### Table 7. Effect of *F. velutipes* mushroom residue (MSR) on muscle tissue antioxidant status in broilers on day 42\(^1\).

| Items              | CON | 1%MSR | 2%MSR | SEM  | T    | L    | Q    |
|--------------------|-----|-------|-------|------|------|------|------|
| Thigh muscle       |     |       |       |      |      |      |      |
| SOD (U/mL)         | 12.60| 14.12| 13.48| 0.41 | .443 | .128 | .314 |
| GSH-PX (U/mL)      | 59.53| 60.66| 65.53| 1.97 | .013 | .011 | .041 |
| CAT (U/mL)         | 10.70| 12.24| 12.24| 0.42 | .015 | .005 | .013 |
| MDA (nmol/ml)      | 1.65| 1.39  | 1.41  | 0.07 | .005 | .001 | .002 |
| Breast muscle      |     |       |       |      |      |      |      |
| SOD (U/mL)         | 10.12| 12.54| 13.88| 0.44 | .057 | .186 | .001 |
| GSH-PX (U/mL)      | 55.66| 58.23| 63.04| 1.79 | .004 | .013 | .002 |
| CAT (U/mL)         | 10.87| 11.81| 12.44| 0.57 | .047 | .034 | .038 |
| MDA (nmol/ml)      | 1.36| 1.28  | 1.17  | 0.06 | .005 | .002 | .002 |

\(^1\)Data represented the mean value of 7 samples per treatment. CON: control; MSR: *Flammulina velutipes* mushroom stem residue; T-AOC: total antioxidant capacity; SOD: superoxide dismutase; GSH-PX: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde. SEM: pooled standard error of the means; T, overall effect of treatment; L, linear effect of increasing MSR; Q, quadratic effect of increasing MSR (1% and 2% diet), a,b, – means in the same row with different letters are significantly different at \(p < .05\).
offered MSR at both levels, compared with control (CON). The value for malondialdehyde (MDA), was lower (linear and quadratic, \( p < .05 \)) in broilers offered MSR diets of both thigh and breast muscle samples than CON. However, total antioxidant capacity (T-AOC) was found higher (quadratic, \( p < .05 \)) than CON (Table 8). Malondialdehyde (MDA) level was lower (linear and quadratic, \( p < .05 \)) in MSR diets compared with CON at the both evaluating periods. Triglyceride (TG) value was lower (linear and quadratic, \( p < .05 \)) in broilers offered MSR diets compared with CON at the both evaluating periods.

**Liver tissue antioxidant status**

On day 21, liver tissue superoxide dismutase (SOD) value, glutathione peroxidase (GSH-PX) value, and catalase enzyme activity (CAT) value were higher (linear and quadratic, \( p < .05 \)) in broilers offered 2% MSR than CON (Table 8). Malondialdehyde (MDA) level was lower (linear and quadratic, \( p < .05 \)) in broilers offered MSR at both levels compared with CON.

On day 42, liver tissue SOD value was significantly higher (linear and quadratic, \( p < .05 \)) in 2% MSR diet than CON. GSH-PX value and CAT value were higher (linear and quadratic, \( p < .05 \)) in MSR groups compared with CON. MDA value was lower (linear and quadratic, \( p < .05 \)) in both levels of MSR diets than CON. Although, T-AOC in liver sample was not affected in this study, but the value was non-significantly higher (\( p > .05 \)) in MSR diets compared with CON.

**Liver tissue lipid status**

Lipid profile in liver tissue sample of experimental broilers is shown in Table 9. Total cholesterol (TC) content of liver tissue was lower (linear and quadratic, \( p < .05 \)) in 2% MSR compared with CON at the both

### Table 8. Effect of *F. velutipes* mushroom residue (MSR) on liver tissue antioxidant status in broilers.

| Items           | CON  | 1%MSR | 2%MSR | SEM  | T  | L  | Q  |
|-----------------|------|-------|-------|------|----|----|----|
| Day 21          |      |       |       |      |    |    |    |
| T-AOC (U/mL)    | 13.72| 16.72 | 15.07 | 0.95 | .148| .069| .190|
| SOD (U/mL)      | 38.13| 46.94b| 59.57b| 2.93 | .036| .012| .033|
| GSH-PX (U/mL)   | 0.30b| 0.32b | 0.43a | 0.03 | .040| .026| .016|
| CAT (U/mL)      | 31.12b| 41.58b| 50.42a| 2.18 | .008| .006| .026|
| MDA (nmol/mL)   | 4.01a | 2.19b | 2.04b | 0.32 | .048| .013| .041|
| Day 42          |      |       |       |      |    |    |    |
| T-AOC (U/mL)    | 5.62 | 6.22  | 6.48  | 0.38 | .916| .470| .773|
| SOD (U/mL)      | 31.36b| 35.45b| 38.93a| 1.08 | .038| .003| .013|
| GSH-PX (U/mL)   | 0.17b| 0.22a | 0.23a | 0.02 | .032| .019| .045|
| CAT (U/mL)      | 9.03b | 16.29a| 20.01a| 1.75 | .040| .005| .018|
| MDA (nmol/mL)   | 2.80a | 1.22b | 0.93b | 0.19 | .040| .008| .035|

1Data represented the mean value of 7 samples per treatment. CON: control; MSR: *Flammulina velutipes* mushroom stem residue; T-AOC: total antioxidant capacity; SOD: superoxide dismutase; GSH-PX: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde. SEM: pooled standard error of the means; T: overall effect of treatment; L: linear effect of increasing MSR; Q, quadratic effect of increasing MSR (1% and 2% diet). a,b, – means in the same row with different letters are significantly different at \( p < .05 \).

### Table 9. Effect of *F. velutipes* mushroom stem residue (MSR) on liver tissue lipid status in broilers.

| Items        | CON  | 1%MSR | 2%MSR | SEM  | T  | L  | Q  |
|--------------|------|-------|-------|------|----|----|----|
| Day 21       |      |       |       |      |    |    |    |
| TC (mmol/g)  | 1.24a| 0.90b | 0.77b | .08  | .048| .007| .026|
| TG (mmol/g)  | 1.16a| 0.88b | 0.62b | .11  | .047| .017| .035|
| Day 42       |      |       |       |      |    |    |    |
| TC (mmol/g)  | 0.73a| 0.59b | 0.52b | .03  | .030| .003| .013|
| TG (mmol/g)  | 0.82a| 0.63b | 0.60b | .04  | .007| .004| .016|

1Data represented the mean value of 7 samples per treatment. CON: control; MSR: *Flammulina velutipes* mushroom stem residue; TC: total cholesterol; TG: triglyceride. SEM: pooled standard error of the means; T: overall effect of treatment; L: linear effect of increasing MSR; Q, quadratic effect of increasing MSR (1% and 2% diet). a,b, – means in the same row with different letters are significantly different at \( p < .05 \).

**Discussion**

It has been known that the biological components (e.g. phenolic compound, glucan, etc.) present in mushroom that shows the antioxidant function. Conventional uses of different synthetic antioxidants, for example butylated hydroxyanisole and butylated hydroxytoluene may have public health hazard, so it is very necessary to discover natural antioxidant products that can improve the antioxidant capacity of animals and their products. This study did not find any significant differences on growth performance which ensured that feeding mushroom residue had no any adverse effects on normal weight gain in broilers. Those findings were similar in broilers fed with mushroom (*Pleurotus ostreatus*) at 2% level (Daneshmand et al. 2012). In addition, 5% mushroom mycelium (*Flammulina velutipes*) did not improve feed intake in broilers (Lee et al. 2012). Similarly, 2.5% *Flammulina velutipes* stem residue had no any effects on average daily weight gain and gain to feed ration in pigs (Liu et al. 2020). In contrast, higher body weight gain and lower feed gain ration were noted in broilers fed with *Agaricus bisporus* mushroom at 2% level (Giannenas et al. 2010). This differs may due to mushroom species, parts of mushroom, inclusion level, and their application procedure in experimental diets of broilers.

The development of the gastro intestinal tract has a positive role in the normal physiology of birds, and the weight of inner organs is considered as the indicator of sound physiology. Bird’s immune function can be measured via the weight of spleen, liver, thymus, bursa, and lymphoid tissues (Al-Khalifa 2016). The bursa is very important central immunity organ, which
is an indicator of better health status and good physiological response to body immune system in poultry (Liu et al. 2019). Mushroom contains naturally higher levels of crude fibre, which may serve to increase relative bursa weight in present trial. These observations were similar with the past findings, where higher bursa weight was observed in male broilers fed with *Lentinus edodes* mushroom extract (Willis et al. 2007). In addition, the weight of digestive organs can be improved by the addition of insoluble fibre to the diets in birds (Yokhana et al. 2016). The other relative organs weight were not differed via MSR supplementation in our study, which ensured that normal health of broilers was not impaired by consuming MSR during the experimental period. In previous studies, also noted that the inner organs weight were not affected in broilers offered with mushroom stem base diets (Daneshmand et al. 2012; Guimarães et al. 2014).

Manipulating diets by different treatments can improve the meat quality in poultry (Cheng et al. 2019). Lower pH and water-holding capacity increases the losses of soluble nutrients that ultimate leads to poor quality of meat (Ozturk et al. 2012). Drip loss is one of the important parameters to estimate the water-holding capacity of meat (Cheng et al. 2019). No significant differences on meat colour; redness (\(a^*\)) and yellowness (\(b^*\)), in broilers offered *Agaricus brasiliensis* mushroom was previously reported (Guimarães et al. 2014) which was similar with the current findings. In addition, dietary inclusion of mushroom (*Flammulina velutipes*) stem residue had no significant effects on meat colour and pH in growing finishing pigs (Liu et al. 2020). The pH value in our study was within acceptable range and it was considered that slaughtered broiler’s meat have a pH from 5.6 to 5.9 (Schilling et al. 2008; Guimarães, et al. 2014). pH is a good indicator of meat quality due to its declining rate is associated to meat tenderness (Lonergan et al. 2010; Guimarães, et al. 2014). In contrast with Shang et al. (2014) have stated the positive effects of meat pH at 24h (\(pH_{24h}\)), drip loss, colour value with fermentation concentrate of *Hericium caput-medusae* mushroom in broilers. These variable results about the effect of mushroom may be due to different species, level of supplementation, composition of diets and interaction with other dietary additives. Our study suggested that dietary inclusion of MSR up to at 2% level does not affect the overall meat quality in broilers. It was probably the lower levels of MSR supplementation in diets. However, to our knowledge, it is the first experimental reporting about *Flammulina velutipes* mushroom residue as dietary supplement on meat quality in broilers. Therefore, we suggest to further study with MSR on meat quality of broilers.

Releases of free radicals by oxidation are common body metabolites in the host organisms. Antioxidants are known to inhibit oxidation and decrease free radicals in body system (Liu et al. 2014). This study assumed that mushroom contains different bioactive components especially phenolic compound, that may have a role on increasing the value of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT) and lowering malondialdehyde (MDA) values in serum, muscle and liver sample of broilers fed with MSR. Shang et al. (2016) reported that SOD, GSH-PX and CAT values were increased, while the MDA level was decreased in the serum, liver, and breast muscle with the mushroom (*Hericium caput-medusae*) inclusion in diets of broilers. Similarly, dried mushroom exerts (*Agaricus bisporus*) could reduce the malondialdehyde in the liver, breast and thigh tissues, whereas it could increase the synthesis of glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase in broilers offered mushroom diets than control (Giannenas et al. 2010). In a recent study reported that dietary inclusion of dried mushroom (*Agaricus bisporus*) stem residue could increase the level of SOD and GSH-PX in serum of laying hens (Yang et al. 2020). Furthermore, antioxidant genes expression (mRNA) in the nuclear factor erythroid 2-related factor 2 (Nrf2) and superoxide dismutase-1 (SOD-1) were found to be higher in broilers offered with mushroom stalk residue and with mushroom waste compost diets (Chuang et al. 2020; Hsieh et al. 2020). *Flammulina velutipes* contains high phenolic amount that exhibit highest antioxidant functions (Zeng et al. 2012; Rahman et al. 2015). In addition, the polysaccharides and oligosaccharide presence in *Flammulina velutipes* mushroom shows the antioxidant function (Ma et al. 2015; Xia 2015). Besides, *Flammulina velutipes* mushroom was found to exhibit vitamin-C and selenium that may play a role on antioxidant function (Tang et al. 2016).

We found that MSR had a positive role on reducing total cholesterol (TC) and triglyceride (TG), in broilers. Mushroom stem residue in experimental diets contained higher fibre that could reduce cholesterol concentration via improving lipid metabolism in broilers. The present results were similar with Shang et al. (2014) who noted that broilers offered *Hericium caput-medusae* mushroom could reduce the production of cholesterol in meat. Broilers offered *Agaricus blazei* powder at 2 g/kg has been recorded to reduce
cholesterol levels in serum (Daneshmand et al. 2012; Fanhani et al. 2016). Furthermore, lowest serum TG concentration was found in chicken fed with oyster mushroom than the control diet (Toghyani et al. 2012). In a recent study reported that dietary Flammulina velutipes mushroom waste compost supplementation could induce all adipolysis related mRNA and up-regulated adipolysis in broilers (Chuang et al. 2020). Edible mushrooms have been suggested to use as oral medicine due to its hypo-cholesterolemic effects on health (Sun et al. 2007). Different metabolic disorders in poultry such as fatty liver and higher abdominal fat are correlated with high levels of TG and LDL in serum or other tissue (Leeson et al. 1995). Our research also suggests that mushroom stem residue can be used on improving the cholesterol metabolism in broiler diets.

Conclusions
This research highlighted that Flammulina velutipes mushroom stem residue could increase the antioxidant enzyme superoxide dismutase, glutathione peroxidase, and catalase value in serum, thigh muscle, breast muscle and liver sample of broilers. Also the test diets could decrease malondialdehyde content in serum, thigh muscle, breast muscle, and liver tissue of broilers. Moreover, mushroom stem residue could decrease total cholesterol and triglyceride concentration of liver sample in broilers. Thus, mushroom stem residue at 2% level can be applied in broilers on improving the antioxidant status, lipid metabolism as well as for useful utilisation of agricultural waste and to reduce the environmental pollution.

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Ethical approval statement
The experiment was approved by Animal Care and Use Committee of China Agricultural University (Beijing, China, CAU-XSPLAB-B-2019-03).

Disclosure statement
There is no conflict of interest relevant to this publication.

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