Dietary *Yucca schidigera* extract improved growth performance and liver antioxidative function in broilers

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**ABSTRACT**

In this study, we tested the effects of *Yucca schidigera* extract (YSE) on the growth performance in broilers, and furthermore, its effects on antioxidative enzyme activities and corresponding gene in the liver of broilers. A total of 128 14-day-old broiler chickens were randomly assigned to four treatments: maize-soybean meal as the basal control diet and the basal diet containing 100, 200, or 300 mg/kg of YSE, respectively, in this study. Each treatment was consisted of four replicate pens with eight broilers per pen. The experiment lasted 28 days. Average daily gain (ADG), average feed intake (AFI) and feed efficiency (FE) were recorded during grower period (d 15 to d 28) and finisher period (d 29 to d 42), respectively. On day 28 and 42, liver samples were collected to analyse superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities, malondialdehyde (MDA) concentrations, total antioxidant capacity (T-AOC), and gene expressions of SOD, CAT, GPx. The results showed that during grower period, there was no difference on growth performance, while CAT activity and its gene expression were increased at 200 mg/kg YSE level. During finisher period, 100 mg/kg YSE supplementation enhanced ADG, and 100 and 200 mg/kg YSE groups improved FE. T-AOC was improved at both 200 and 300 mg/kg. 300 mg/kg supplementation of YSE enhanced GPx and SOD activity, and decreased MDA concentration. GPx gene expression was up-regulated at 300 mg/kg level. In conclusion, YSE promoted growth performance in broilers as well as exhibited liver antioxidative ability during finisher period.

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**Introduction**

Oxidative stress is caused by increased ROS (reactive oxygen species) in cells. The major ROS in cells are superoxide radical anion (*O*$_2^\cdot$), hydrogen peroxide (H$_2$O$_2$) and the highly reactive hydroxyl radical (OH$^*$) (Sen et al. 2010). Several antioxidative enzymes in cells act as the defence system to prevent the formation of reactive species such as catalase (CAT), superoxide dismutase (SOD) and glutathion peroxidase (GPx) (Sanders et al. 2004).

Modulating dietary treatments of livestock such as adding antioxidants is one of the effective means to relieve oxidative stress potentials among various methods (Sen et al. 2010). *Yucca schidigera* (YS), prevalent in the deserts of the south-western United States and northern Mexico, is considered highly for its pharmaceutical values (Cheeke et al. 2006; Patel 2012). *Yucca schidigera* extract (YSE) was applied as dietary additives for livestock primarily for ammonia and odour control due to the presence of saponin (Cheeke 2000; Piacente et al. 2005; Ayasan 2013). But two other main active components, resveratrol and yuccaols, which possess biological functions, were identified in YS besides steroidal saponins (Patel 2012). Resveratrol is well known to be an effective scavenger of hydroxyl, superoxide radicals, as well as inhibiting ROS formation in cells. It also protects cell from lipid peroxidation in membranes and DNA damage caused by ROS (Leonard et al. 2003). Phenolic constituents such as yuccaols in YS which structurally related to resveratrol, also possess radical scavenging activity (Piacente et al. 2004, 2005; Patel 2012).

Alagawany et al. (2016) reported that YSE supplementation improved SOD and reduced glutathione (GSH) level, and reduced MDA concentration in serum of laying hens. Another experiment observed increased GPx and CAT in rabbits with YSE supplementation (Ashour et al. 2014). Ince et al. (2013) demonstrated...
that dietary YSE incorporation, in a dose-dependent manner, ameliorated arsenic-induced oxidative stress, lipid peroxidation, and increased antioxidant enzyme activities in mice. In another study, YSE treatment to rats was found to decrease blood and tissue malondialdehyde, and increased the glutathione in blood and various tissues (Cigerci et al. 2009).

Many reports have also reported that dietary YSE incorporation could produce positive effects on the average weight (Sahoo et al. 2015), FE (Ayasan et al. 2005; Wang & Kim 2011), behaviour (Sahoo et al. 2015) and health of chicks (Alfaro et al. 2007). However, there was little information on that using YSE as a feed additive to ameliorate the potential negative effects of oxidative stress in broilers. The objective of the present study was to elucidate the effects of YSE on growth performance, liver antioxidant status in broilers, and find out whether they are related to each other.

Materials and methods

Ethics statement

The experiment was carried out in a poultry research facility located at the Inner Mongolia Agricultural University, Hohhot, China. All experimental procedures performed were approved by the Animal Research and Ethics Board of College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China.

Animals, experiment design and treatments

One-day-old AA broilers were purchased from a commercial farm in Hohhot, Inner Mongolia, China. They were housed in electrically heated battery brooders until day 7 and transported to the experimental site until day 14. Then, 128 14-day-old chickens of uniform body weight were selected and were reared in stainless-steel wire cages. Their average initial body weight was 372.82 ± 6.35 g. Broilers were assigned to 4 dietary treatments. Each treatment was randomly divided into 4 equal replicates, with 8 chickens/cage (100 × 50 × 50 cm). The duration of experimental period was 28 days, and was divided into grower period (d 15 to d 28) and finisher period (d 29 to d 42). Experimental diets and water were available ad libitum during the entire experimental period. Before the experiment, the poultry facility was fumigated using methanal plus potassium permanganate to disinfect the environment. Poultry facilities had thermostatically controlled heater and lighting programme. Pens were equipped with a pan feeder, a manual drinker. Drinkers were daily washed to prevent faecal and microbial contamination.

YSE product and diets

YSE powder was purchased from Shanxi Yuanzhixing Biotechnology Co., Ltd (Xian, China). Steroidal sapogenin content of YSE was >7%. Diets were formulated to meet nutrients recommendation of Feeding Standard of Chicken, China (NY/T 33-2004) (Chinese Ministry of Agriculture 2004). Diets were fed in mash form. Diet treatments were as follows: (1) control diet (basal diet without supplement), (2) basal diet with 100 mg/kg of YSE, (3) basal diet with 200 mg/kg of YSE, and (4) basal diet with 300 mg/kg of YSE. The formulation and composition of the control diet is found in Table 1.

Growth performances and sample collection

Average daily gain (ADG), average feed intake (AFI) were recorded during grower period and finisher period, and feed efficiency (FE) was calculated as feed intake/body weight gain. On day 28 and day 42, 2 chickens from each pen (8 per treatment) were randomly selected, weighed, stunned, and slaughtered by exsanguination. And liver tissues were collected based on the measures as described by Tufarelli et al. (2016), and the samples were stored at −80°C to measure the antioxidative parameters and gene expression.

Antioxidative enzyme activities

All reagents for these assays were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

Table 1. Feed composition and nutrient content of the basal experimental diet.

| Ingredients        | Grower (d 14 to 28) | Finisher (d 29 to 42) |
|--------------------|---------------------|-----------------------|
| Maize              | 51.68               | 58.49                 |
| Soybean meal, CP44%| 41.00               | 34.30                 |
| Soybean oil        | 3.00                | 3.00                  |
| Dicalcium phosphate| 1.90                | 1.80                  |
| Limestone          | 1.10                | 1.20                  |
| NaCl               | 0.37                | 0.37                  |
| Lysine             | 0.05                | 0.03                  |
| Methionine         | 0.19                | 0.10                  |
| Premix*            | 0.71                | 0.71                  |
| Total              | 100.00              | 100.00                |
| Chemical composition |                    |                       |
| ME, kcal/kg        | 3018                | 3078                  |
| Crude protein, %   | 21.84               | 19.95                 |
| Calcium, %         | 1.00                | 1.00                  |
| Available phosphorus, % | 0.48           | 0.46                  |
| Lysine, %          | 1.40                | 1.20                  |
| Methionine, %      | 0.56                | 0.44                  |
| Dicysteine, %      | 0.40                | 0.37                  |

ME: metabolisable energy.

*Contents per kg premix: Fe 60.91 mg; Cu 6.01 mg; Zn 65.75 mg; Mn 62.3 mg; I 0.9 mg; Se 0.21 mg; vitamin A 6141.5 IU; vitamin D3 1789.2 U; vitamin E 7.99 U; vitamin K 1.82 mg; vitamin B6 0.65 mg; vitamin B12 3.93 mg; vitamin B12 2.08 mg; vitamin B12 0.01 mg; niacin 18.06 mg; calcium pantothenate 6.65 mg; folic acid 0.59 mg; biotin 0.07 mg; choline chloride 332.28 mg.
MDA, T-AOC, GPx, T-SOD, and CAT activities in liver samples were measured according to the instructions of manufacture (Cao et al. 2015; Liao et al. 2015).

**Total RNA extraction and reverse transcription**

Total RNA was extracted from liver tissues by Trizol extraction method described by Trizol manufacturer (RNAiso Plus Kit, Takara Bio Inc, Kusatsu, Japan). The quantity and purity of RNA samples was measured by using Nano Drop spectroscopy (Thermo Scientific, Waltham, MA) with the ratio of absorbance at 260 nm and 280 nm (Mueller et al. 2012). Reverse transcription was performed by using a commercial complementary DNA synthesis kit according to manufacturer’s instructions included in the kit (Reverse Transcription System Kit, Takara Bio Inc, Kusatsu, Japan).

**Quantitative real-time PCR**

Primers sequences for β-Actin, SOD, CAT, and GPx were designed by Gene bank database sequences from BGI Tech (Shenzhen, China) corresponding to each quantified gene (Table 2). Quantitative real-time PCR (RT-PCR) was performed by IQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA) on 96-well plate with 20 µl of total reaction volume including 10 µl SYBR Premix Ex Taq TM II (Takara Bio Inc, Kusatsu, Japan), 0.4 µl of forward primer (10 pmol), 0.4 µl of reverse primer (10 pmol), 2 µl of cDNA, and 7.2 µl of nuclease-free water. SYBR Premix Ex Taq TM II was used as RT-PCR master mix and each reaction was run in duplicate. The following PCR programme was employed to amplify target mRNA in tissue extracts: an initial denaturation step at 95°C for 30 s, followed by amplification for 40 cycles at 95°C for 5 s, and 55°C for 20 s, and 72°C for 20 s, and annealing step at 72°C for 7 min, then extension at 95°C for 20 s.

**Statistical analyses**

The results are expressed as mean ± SEM or mean ± SD. The statistical significance of data was evaluated by ANOVA procedure of SAS 9.2 (SAS Institute 2002). Data were analysed by Duncan’s multiple range test. Differences in the mean values were considered significant at $p < .05$, whereas $0.05 < p < .10$ was considered to constitute a tendency.

**Results**

**Growth performance**

The influence of dietary YSE on growth performance in broilers was shown in Figure 1. During finisher period, ADG was increased with 100 mg/kg YSE in the diets ($p = .034$), FE was enhanced at 100 and 200 mg/kg YSE groups ($p = .038$). In the whole experimental period, there was a tendency of the increasing of ADG at 100 mg/kg level ($p = .072$), FE was enhanced at both 100 and 200 mg/kg groups ($p = .011$).

**Antioxidative activities and gene expressions in liver**

The results of antioxidant enzymes activity in liver were shown in Table 3, the corresponding gene expressions were shown in Figure 2. During grower period, CAT activity was increased at 200 mg/kg YSE level, CAT gene expression was up-regulated with 200 mg/kg YSE addition ($p = .003$). MDA concentration was shown a decreasing tendency at 200 mg/kg level. During finisher period, T-AOC was improved at both 200 and 300 mg/kg, with 300 mg/kg group exhibiting the strongest effect. 300 mg/kg supplementation of YSE enhanced GPx and SOD activity, and decreased MDA concentration. GPx gene expression was up-regulated at 300 mg/kg level ($p = .028$).

**Discussion**

**Growth performance**

In the present study, we observed the better ADG and FE in broilers. The optimum dose in our study was in line with a study of Sahoo et al. (2015) indicating that 125 mg/kg YSE increased the growth of broilers in 6th week by utilising lesser FI, better FCR, protein

| Target   | Primers | PCR product | Accession no. |
|----------|---------|-------------|---------------|
| NM_205518 | 118     | S’-GCCAACACAGAGAAGATGACAC-3’ | j-Actin |
| NM_205064.1 | 98    | S’-GTAAACACCATCACCAGGCACGTTGCA-3’ | SOD |
| NM_001031215.1 | 182 | S’-TGCTGTCTTCAGGAGGATGAAAGTGA-3’ | CAT |
| NM_001163245.1 | 136 | S’-CAAGGTGCTCGTCAGGGA-3’ | GPx |

Table 2. Primers used for quantitative real-time PCR analysis of chicken mRNAs.
efficiency ratio, and energy efficiency ratio. However, current study only observed the improvement of growth performance in finisher period, but not in grower period. We proposed that it may be related to different energy requirements of broilers in different phase, thus the different changes of energy with YSE addition, as Kucukkurt & Dundar (2013) indicated that YSE supplementation affects energy metabolism through modulating hormone secretions and depressing energy compounds in the organism.

![Figure 1](image)

**Figure 1.** Effects of YSE on growth performance in broilers. The figure describes effects of dietary YSE on ADG (a), AFI (b), FE (c) in broilers. Statistical comparisons are made between control group and YSE-added groups. Results are expressed as means ± SD. Asterisks indicate significant differences according to different supplementing level of YSE (*p* < .05).

**Table 3.** Effects of YSE on antioxidative functions in liver.

| Items                  | 0          | 100        | 200        | 300        | SEM   | p Value |
|------------------------|------------|------------|------------|------------|-------|---------|
| T-AOC, U/mgprot        |            |            |            |            |       |         |
| Grower                 | 3.761bc     | 4.262ab    | 4.723a     | 4.306ab    | 0.216 | .171    |
| Finisher               | 4.203bc     | 3.473c     | 4.585ab    | 4.838c     | 0.001 | .001    |
| CAT, U/mgprot          |            |            |            |            |       |         |
| Grower                 | 26.797bc    | 30.448b    | 38.149a    | 33.793ab   | 2.007 | .035    |
| Finisher               | 71.093c     | 62.708c    | 73.121c    | 75.234c    | 4.310 | .479    |
| MDA, nmol/mgprot       |            |            |            |            |       |         |
| Grower                 | 1.252a      | 1.164ab    | 1.073b     | 1.285a     | 0.051 | .066    |
| Finisher               | 1.719a      | 1.574a     | 1.574a     | 1.342b     | 0.063 | .006    |
| GPx, U                 |            |            |            |            |       |         |
| Grower                 | 12.367a     | 11.157c    | 12.955a    | 12.423a    | 0.540 | .287    |
| Finisher               | 16.477bc    | 14.885c    | 18.343c    | 19.846c    | 0.880 | .002    |
| T-SOD, U/mgprot        |            |            |            |            |       |         |
| Grower                 | 472.271a    | 515.827ab  | 585.488c   | 512.642ab  | 23.50 | .097    |
| Finisher               | 527.004b    | 516.969b   | 531.838b   | 647.535c   | 28.30 | .049    |

abeMeans within a column that do not share a common superscript are significantly different (*p* < .05).
Steroidal saponins are known as major physiologically active components of YS with broad-spectrum biological properties, and the growth-promoting effects of YSE has long been ascribed to them (Piacente et al. 2005). The positive influence of steroidal saponins on better absorption of nutrients in the intestinal tract (McAllister et al. 1998; Wang & Kim 2011) might be responsible for the better growth performance. However, YS bark contains polyphenols such as resveratrol, yuccaols A to E, which possess antioxidant, free-radical scavenging, and anti-inflammatory abilities (Piacente et al. 2004, 2005; Cheeke et al. 2006). It is hypothesised that YSE may exhibit its growth-promoting effects not only due to its saponin components. An experiment supplementing resveratrol to laying chickens might have supported this hypothesis at least to some extent (Zhang et al. 2014). In such study, the resveratrol level that significantly enhanced ADG fell on 400 mg/kg (Zhang et al. 2014), which is higher than the optimum doses observed in the present study (100 mg/kg YSE). We provided that the lower optimum dose observed in our study might be attributed to the composite effects of both saponin fractions and polyphenols. However, the claims must be scientifically verified with more emphasis on animal data.

Although there exists limited researches exploring toxicity of YSE in the application of animals, detecting of dioscin (Xu et al. 2012), a steroidal-saponin containing plants, provided us an implication of the potential toxic effects of YSE in livestock production if administered not appropriately. Whether or not the non-different performance at 300 mg/kg YSE levels in the present study was linked to the potential detrimental effects remains unknown, which needs to be investigated further more specifically.

**Antioxidative activities and gene expressions in liver**

MDA levels in the liver is proved to be a sensitive indicator of the lipid oxidative tendency (Shafey et al. 2014). We provided that the lower optimum dose observed in our study might be attributed to the composite effects of both saponin fractions and polyphenols. However, the claims must be scientifically verified with more emphasis on animal data.

![Figure 2. Effects of YSE on gene expression in liver of broilers. Statistical comparisons are made between control group and YSE-added groups. Results are expressed as means ± SD. Asterisks indicate significant differences according to different supplementing level of YSE (∗p < .05, **p < .01).](image-url)
SOD is an important substance that exists in various tissues and organisms, and is believed to protect cells from damage caused by superoxide radical (O$_2^*$·) (Kurutas 2016). In vitro study have proved the efficiency of phenolics from bark of YS in decreasing ROS production in blood platelets (Olas et al. 2005). In the current study, the decreases of MDA concentrations and the increases of SOD concentrations might be attributed to the YSE ability in terms of scavenging secondary reactive radicals or preventing formation of superoxide and hydrogen peroxide (Enginar et al. 2006). The results of our experiment showed that MDA concentration in finisher period was higher than grower period, which was in line with results of Liu et al. (2011) who indicated that serum MDA levels at d 42 was higher than at d 21. It is assumed that oxidative damage in the liver might be more severe during finisher period than grower period, and the higher SOD in finisher phase may suggested that adaptive response may enhance the expression of antioxidant enzymes and compounds in response to free radical-mediated lipid peroxidation products (Kurutas 2016). In our study, the dose of YSE where SOD exhibited positive effects in finisher period was higher than grower period. We proposed that when the oxidative status is intensified such as in our cases, the optimum dose of YSE that exhibits antioxidative functions would be higher accordingly. The decreasing but not significant tendency of MDA during grower period may suggest that protective mechanisms in liver such as hepatic microsomes which have the ability of generating and degenerating TBARS (Venkatraman et al. 1998) expressed certain function to maintain MDA towards normal levels, or due to the different producing site of lipid peroxides as described in the article of Shafey et al. (2015).

CAT catalyses the conversion of hydrogen peroxide to water and oxygen (Kurutas 2016). GPx acts as the catalyst to transform reduced glutathione (GSH) and H$_2$O$_2$ into oxidised glutathione (GSSG) and H$_2$O (Kurutas 2016). We noticed that CAT was only promoted at grower period, while GPx was enhanced at finisher period but not in grower period. This demonstrated that in different phase, YSE might possess the ability to eliminate H$_2$O$_2$ primarily by either enhancing CAT or GPx, since CAT competes with GPx for hydrogen peroxide as a substrate (Molavian et al. 2015).

According to Limón-Pacheco & Gonsebatt (2009), CAT and GPx were not co-existed in the organism. CAT is primarily existed in subcellular organelles such as peroxisomes, while GPx are present in the cytoplasm and mitochondrial matrix. Mitochondria and the endoplasmic reticulum barely contain CAT. It is assumed that H$_2$O$_2$ might be produced in different sites in different growing period of broilers, thus CAT or GPx could serve their own H$_2$O$_2$-eliminating functions. CAT gene expression corresponded well with its enzyme activities in grower period, and GPx expression was in accordance with its enzyme activity in finisher period. These suggested that YSE could participate in the removal of H$_2$O$_2$ by up-regulating CAT expression during grower period and GPx expression during finisher period. In our study, although we did not detect the significant difference, the paralleling tendency between SOD expression and its enzyme activity during grower period gave us a hint of the potential of YSE to up-regulate SOD activity during grower period.

T-AOC, comprised of several key antioxidative enzymes and other mechanisms, is a reflection of total antioxidative ability within the body (Ahmad et al. 2012). In our experiment, YSE supplementation enhanced T-AOC in finisher period, proved that YSE exhibits certain antioxidant ability, and this was at least partially due to the enhancement of SOD and CAT/GPx both at transcript and translation levels based on our results. However, T-AOC was not increased in grower period. The enhancing but not significant tendency of SOD during grower period might contribute to the trend to some extent.

Alagawany et al. (2016) reported that YSE up to 100 mg/kg could increase SOD, glutathione (GSH), and decrease MDA concentrations in laying hens. Another study observed increases of plasma GSH concentrations and T-AOC in 100 ppm YSE group (Aslan et al. 2005). In contrast, our results indicated that higher doses of YSE supplementation improved antioxidative functions in the liver of broilers. This inconsistency might be attributed to the physiological differences between broilers and laying hens. In our earlier study, the dose that stimulates growth and immune functions lies on 100 mg/kg level (Su et al. 2016), which was lower than optimum doses that express antioxidative functions, which resembles to yuccaols in structures (Piacente et al. 2005), rapid sulfatation and glucuronidation by theintestine seem to limit its systemic bioavailability (Walle et al. 2004). In addition, phenolics only occur in the external part of the trunk, not inside (Piacente et al. 2005). Thus, a margin of safety above...
the estimated requirements, as well as the economic efficiency, should be considered if the present data are used as a basis for formulation of broiler diets. Further tests need be focused on the effects of bark part of YSE on the antioxidative capacities of broilers.

Conclusions
In conclusion, 100 and 200 mg/kg dietary YSE promoted growth performance of broilers, while 200 and 300 mg/kg dietary YSE enhanced antioxidant capacities in the liver. And the effectiveness of YSE on growth performance and antioxidant system was mostly expressed during finisher period. The enhanced antioxidative capacities in liver may contribute to the better growth performance in broilers.

Implications
There are now studies available combining YSE with other additives such as caprylic acid (Wang & Kim 2011; Begum et al. 2015), coccidiostat (Alfaro et al. 2007), zeolite (Çabuk et al. 2004), yeast cell walls (Gurbuz et al. 2011), or coccidiosis vaccine (Alfaro et al. 2007), to explore their potentials in improving the performance of livestock. Our results showed that YSE alone exhibited greatest potentials in the finisher phase of broilers, which gives us a cue to explore the combining effects of YSE and other additives, to see if they could improve the productivity of animals in a wider range of time.

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Disclosure statement
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