



Effect of *Ginkgo biloba* Leaf Powder on Growth Performance, Meat Quality and Antioxidant Activity of Muscle in Growing-Finishing Pigs

Xianglun Zhang, Peng Lu, Wenyue Xue, Dawei Wu, Chao Wen, Liren Ding and Yanmin Zhou*

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

ABSTRACT

An experiment was conducted to evaluate the effect of *Ginkgo biloba* (*G. biloba*) leaf powder on growth performance, meat quality and antioxidant activity of muscle in growing-finishing pigs. A total of 75 finishing barrows were randomly distributed to 5 groups, receiving basal diet (Control), basal diet supplemented with 0.50% coarse *G. biloba* leaf powder (CG) or basal diet supplemented with 0.05% (UG1), 0.25% (UG2) and 0.50% (UG3) ultrafine *G. biloba* leaf powder, respectively. Meat quality of longissimus dorsi muscle and leg muscle was improved by addition of UG, which was consistent with the simultaneously enhanced antioxidant capacity of muscles. Additionally, pigs fed diet supplemented with CG also exhibited improved antioxidant status of measured muscles than that of control. The antioxidant capacity of both longissimus dorsi muscle and leg muscle of pigs fed CG and UG especially the UG were better than that of the control. In conclusion, addition of *G. biloba* leaf powder resulted in improvement in meat quality and antioxidant capacity of muscle and this effect was more pronounced when supplementing ultrafine *G. biloba* leaf powder.

Article Information

Received 5 May 2015

Revised 20 October 2015

Accepted 27 March 2016

Available online 1 August 2016

Authors' Contribution

YZ conceived and designed the study. XZ wrote the article. PL, WX, DW executed the experimental work. YZ and CW supervised the study.

Key words

G. biloba leaf powder, Pig, Growth performance, Meat quality, Antioxidant activity

INTRODUCTION

As a traditional natural medicinal herb in China, *G. biloba* is a sole living member of the family *Ginkgoaceae* that has survived unchanged for millions of years (Jacobs and Browner, 2000). The extract from *G. biloba* leaf has been utilized in many fields such as food, biochemistry, as well as in pharmacology, showing high antioxidant activity (Goh *et al.*, 2003; Kobus-Cisowska *et al.*, 2014), antibacterial effect (Lee *et al.*, 2014), and physiological activity in therapies for diseases (Puebla-Pérez *et al.*, 2003; Lu *et al.*, 2006). Chemically, the active constituents of the *G. biloba* leaf are flavonoids (flavone glycosides, primarily composed of quercetin) and terpenoids (ginkgolides and bilobalides) (Pietri *et al.*, 1997; van Beek and Montoro, 2009). *G. biloba* extract also shows high biological activity when fed to animal. Yao *et al.* (2006) observed that *G. biloba* extract could provide protection against ethanol-induced oxidative stress on various tissues of male rats. Şener *et al.* (2007) also found that *G. biloba* extract could protect many tissues of rat from suffering oxidative stress induced by mercury (II).

G. biloba extract is prepared in a multistep process (van Beek and Montoro, 2009) that may cause a higher

price and many nutrients in *G. biloba* leaf may be damaged during extraction process. Dietary addition of *G. biloba* leaf directly may reduce the cost and protect the nutrients in the leaf. At present, there are few researches on the application of *G. biloba* leaf in the animal production (Cao *et al.*, 2012; Zhang *et al.*, 2012; Yu *et al.*, 2015). The addition level of *G. biloba* leaf in the feed of the above studies ranges from 0.35% to 0.70%, but the particle size of *G. biloba* leaf is not definite. Particle size may be an important factor (Fastinger and Mahan, 2003; Li *et al.*, 2008) affecting the bioavailability of *G. biloba* leaf. Coarse grinding cannot thoroughly break up the cell walls of *G. biloba* leaf and thus active components in the cell may be difficult to release. Ultrafine grinding technology is a useful tool for making ultrafine powder with good surface properties like dispersibility and solubility (Tkacova and Stevulova, 1998), and have found applications in some fields (Cordeiro *et al.*, 2009; Ma *et al.*, 2009). The reduction of particle sizes of various materials to micro- or nanosizes brings some new outstanding characteristics including surface effect, quantum effect, magnetic property, mechanical property, chemical and catalytic properties that bulk materials do not possess before (Zhu *et al.*, 2010). Our findings have demonstrated that the dissolution of total flavonoids and terpenoids in ultrafine *G. biloba* leaf powder was higher than that in coarse *G. biloba* leaf powder (Zhang *et al.*, 2014). Based on these characteristics, ultrafine *G. biloba* leaf powder may be more effective when added to animal diets. However,

* Corresponding author: zhouym6308@163.com
0030-9923/2016/0005-1555 \$ 8.00/0
Copyright 2016 Zoological Society of Pakistan

little information is available concerning the effects of ultrafine *G. biloba* leaf powder in animal production.

The present work was carried out to investigate the effect of *G. biloba* leaf powder on growth performance, meat quality, meat composition and antioxidant capacity of muscle in growing-finishing pigs.

MATERIALS AND METHODS

Preparation of *G. biloba* leaf powder

Air-dried *G. biloba* leaves were purchased from a local company and were used for this study. The dry matter, crude protein and crude ash compositions of these leaves are 960.0 ± 1.16 , 135.1 ± 0.24 and 136.7 ± 0.19 g/kg, respectively. The leaves were ground into two particle sizes categorised as coarse and ultrafine *G. biloba* leaf powder. The coarse *G. biloba* leaf powder was processed by a water type pulverizer (SDHM, Wuxi Zhongya Food Factory, Wuxi, Jiangsu, China) while the ultrafine *G. biloba* leaf powder was obtained by a micronizer (LHJ-500, Zhengyuan Powder Engineering Equipment Company, Weifang, Shandong, China). The particle sizes of *G. biloba* leaf powder were determined by using a laser Mastersizer (Instruments 2000, UK) according to the Particle size analysis Standard (GB/T 19077.1-2008) of China. The particle sizes of the coarse and ultrafine *G. biloba* leaf powder were: $D_{50} = 560 \mu\text{m}$ and $D_{50} = 18.1 \mu\text{m}$, respectively. The dissolution of flavonoids and terpenoids of the *G. biloba* leaf powder were measured by spectrophotometric method at 510 nm and 514 nm using *Rutin* and *Ginkgolide A* (Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) as standard, respectively. The dissolution of flavonoids of the coarse and ultrafine *G. biloba* leaf powder were: $1.47 \pm 0.06\%$ and $2.00 \pm 0.06\%$, and the dissolution of terpenoids were $0.63 \pm 0.07\%$ and $0.96 \pm 0.10\%$, respectively (Zhang *et al.*, 2014).

Animals and management

The experimental design and procedures were approved by the Animal Care and Use Committee of Nanjing Agricultural University.

Seventy-five [(Landrace Yorkshire) Duroc] finishing barrows with initial body weight of 67.81 ± 1.33 kg were randomly divided into 5 dietary treatments consisting of 3 replicates of 5 pigs each, balanced for body weight with a randomized complete block design. All pigs were provided with mash feed and water *ad libitum* and were reared in collective cages with solid concrete floor. The rearing period was 45 d.

Pigs in the control group were fed a basal diet, and pigs in the other four groups were fed the basal diet supplemented with 0.50% coarse *G. biloba* leaf powder,

0.05%, 0.25% and 0.50% ultrafine *G. biloba* leaf powder, referred to as CG group, UG1 group, UG2 group, and UG3 group, respectively. Pigs used in the study were all adapted to the experimental diets for 7 d before the experiment started. The compositions of the basal diets and nutrient levels were formulated according to the NRC (1998) nutrient requirements (Table I).

Table I.- Ingredients and nutrient composition of basal diet, air dry basis.

| Ingredient | % | Calculated nutrient level | |
|---------------------------------------|--------|---------------------------|-------|
| Maize | 67.52 | Digestible energy (MJ/kg) | 13.64 |
| Distillers dried grains with solubles | 10.45 | Crude protein, % | 13.71 |
| Soybean meal | 4.48 | Calcium, % | 0.50 |
| Rice bran | 2.59 | Available phosphorus, % | 0.23 |
| Maize germ cake | 3.98 | Lysine, % | 0.80 |
| Wheat middling | 3.98 | Methionine + cysteine, % | 0.49 |
| Soybean oil | 1.00 | | |
| Soybean phospholipids | 1.00 | | |
| Premix ¹ | 5.00 | | |
| Total | 100.00 | | |

¹Supplied per kilogram of diet: vitamin A acetate, 4800 IU; vitamin D₃, 1000 IU; vitamin E, 20 IU; vitamin K₃, 3 mg; vitamin B₂, 5 mg; niacin, 24 mg; calcium pantothenate, 16 mg; vitamin B₆, 1.5 mg; biotin, 0.05 mg; folic acid, 0.3 mg; vitamin B₁₂ 8 µg; choline, 400 mg; Fe, 100 mg; Zn, 100 mg; Cu, 10 mg; Mn, 12.5 mg; I, 0.5 mg; Se, 0.3 mg.

Growth performance

Body weights were recorded for each replicate at the first day and 45 d of the feeding period. Feed intake was also recorded during the 45-d trial. The body weight gain, feed intake and gain:feed ratio were calculated.

Slaughter and sample collection

At 45 d of the feeding period, 3 pigs in each group (one pig per replicate) were randomly selected and weighed after feed deprivation for 12 h. Pigs were transported to the abattoir where they were humanely sacrificed. After killing, longissimus dorsi muscle and hind leg muscle samples were immediately excised from each carcass for the determination of meat quality. Muscle samples were taken and then stored at -20°C for the assay of meat composition and antioxidant indices.

Meat quality

Longissimus dorsi muscle and hind leg muscle were used to determine pH, objective color measurements, drip loss and cooking loss. The pH at 45 min postmortem (pH_{45 min}) and 24 h postmortem (pH_{24 h}) were measured by a portable pH meter (HI9025, Hanna Instruments,

Italy) according to Schilling *et al.* (2008). Color measurements were taken at 45 min and 24 h postmortem. Lightness (L^*), redness (a^*), and yellowness (b^*) values were determined using a Minolta CR410 chroma meter (Konica Minolta, Tokyo, Japan) as described by Cao *et al.* (2012). Drip loss at 24 h and 48 h postmortem and cooking loss were measured using the methods outlined by Franco *et al.* (2009, 2014).

Meat composition

Meat samples were prepared for determination of moisture, crude protein, ether extract and crude ash based on Association of Official Analytical Chemists procedures (AOAC, 1990).

Assay of antioxidant indices in muscle

Muscle samples were analyzed for total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, the activity of T-SOD was determined by following the nitrite method as described by Oyanagui (1984). One unit of SOD was defined as the amount of SOD required to produce 50% inhibition of the rate of nitrite production at 37 °C. The methodology used in determining GSH-Px activity was dithio-nitro benzene method described by Hafeman *et al.* (1974). One unit of GSH-Px activity was defined as the amount of enzyme that would catalyze the conversion of 1 $\mu\text{mol/L}$ of GSH to oxidized GSH at 37 °C in 5 min. The concentration of MDA was determined by following the TBA method as described by Placer *et al.* (1966). The activities of antioxidant enzymes and the content of MDA were expressed as units per milligram of protein for muscle.

Statistical analysis

The effects of dietary treatment were statistically analyzed by one-way ANOVA (SPSS 16.0). Differences among treatments were examined by Duncan's multiple range test. Values in the tables were means and pooled SEM. Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

Addition of *G. biloba* leaf powder had no significant effect ($P > 0.05$) on growth of pigs, but numerically increased the final weight and body weight gain as compared with that of the control pigs (Table II). All pigs appeared healthy and no mortality occurred during the experimental period (data not shown). Our

findings were similar with Zhang *et al.* (2012) who reported that dietary addition of 0.35% *G. biloba* leaf did not affect the body weight gain and feed conversion compared with the control group in the period of 0 to 21 day of broilers.

Meat quality

The data of meat quality were summarized in Table III and Table IV. The $\text{pH}_{45 \text{ min}}$ of longissimus dorsi muscle in the UG2 group was higher ($P < 0.05$) than that of the control group. No difference ($P > 0.05$) was found in $\text{pH}_{24 \text{ h}}$ of neither longissimus dorsi muscle nor leg muscle among groups, but the $\text{pH}_{24 \text{ h}}$ of both kinds of muscles in UG2 group tended to be the highest numerically. Animal muscle pH is neutral before slaughter, but the pH declines after slaughter because glycolysis causes lactic acid accumulation in muscles (Bai *et al.*, 2013). The results in this trial indicated that dietary addition of *G. biloba* leaf powder might maintained a relatively higher pH value of meat. Muscle pH is highly correlated with numerous meat quality attributes, such as water-holding-capacity, meat color and so on (Huff-Lonergan *et al.*, 2002). The 24-h drip loss of longissimus dorsi muscle in both the UG2 and UG3 groups was lower ($P < 0.05$) than that of the control group. In addition, lower ($P < 0.05$) 24-h drip loss of leg muscle was found in both the CG and UG2 groups. The water-holding-capacity (drip loss and cooking loss) of meat is related to the intramuscular lipids, moisture content and lipid peroxide contents in the muscle (Macit *et al.*, 2003; Cao *et al.*, 2012). The improvement in drip loss might result from the antioxidant capacity of flavonoids and terpenoids in *G. biloba* leaf powder which could alleviate lipid oxidation, enhance integrity of cellular membrane, thus decreased drip loss in muscle (Rong *et al.*, 1996; Kobus-Cisowska *et al.*, 2014). The $a^*_{45 \text{ min}}$ of longissimus dorsi muscle in the UG groups was higher ($P < 0.05$) than that of the control group. Meanwhile, pigs fed with *G. biloba* leaf powder supplemented diets had higher ($P < 0.05$) $a^*_{45 \text{ min}}$ of leg muscle. The results suggested that dietary addition of *G. biloba* leaf powder could improve the color of the meat. Overall, these improvements in meat quality in both longissimus dorsi muscle and leg muscle might result from the antioxidant capacity of flavonoids and terpenoids in *G. biloba* leaf powder. Moreover, UG groups tended to be better in terms of improving meat quality than CG group. This may be due to the better solubility and dispersibility of the ultrafine *G. biloba* leaf powder (Tkacova and Stevulova, 1998) and thus the active components in the cell were easy to release.

Meat composition

The effects of *G. biloba* leaf powder on meat

Table II.- Growth performance of pigs fed diets supplemented with *G. biloba* leaf powder.

| Item | Treatments ¹ | | | | | SEM ² | P-value |
|-----------------------|-------------------------|--------|--------|--------|--------|------------------|---------|
| | Control | CG | UG1 | UG2 | UG3 | | |
| Initial weight (kg) | 67.08 | 67.48 | 67.70 | 68.38 | 68.40 | 1.33 | 0.99 |
| Final weight (kg) | 102.60 | 103.18 | 104.53 | 105.60 | 104.18 | 1.30 | 0.97 |
| Body weight gain (kg) | 35.51 | 35.69 | 36.83 | 37.22 | 35.80 | 0.51 | 0.83 |
| Feed intake (kg) | 120.55 | 115.35 | 119.12 | 116.70 | 113.78 | 1.84 | 0.83 |
| Gain : feed ratio | 0.29 | 0.31 | 0.31 | 0.32 | 0.32 | 0.01 | 0.78 |

¹Control, basal diet; CG, basal diet with 0.50% coarse *Ginkgo biloba* leaf powder; UG1, UG2, and UG3, basal diet with respectively 0.05%, 0.25% and 0.50% ultrafine *Ginkgo biloba* leaf powder.

²SEM, standard error of the mean based on pooled estimate of variation.

Table III.- Meat quality of longissimus dorsi muscle in pigs fed diets supplemented with *G. biloba* leaf powder.

| Item ¹ | Treatments ² | | | | | SEM ³ | P-value |
|----------------------|-------------------------|---------------------|--------------------|--------------------|--------------------|------------------|---------|
| | Control | CG | UG1 | UG2 | UG3 | | |
| pH _{45 min} | 6.24 ^b | 6.42 ^{ab} | 6.41 ^{ab} | 6.68 ^a | 6.42 ^{ab} | 0.05 | 0.04 |
| pH _{24 h} | 6.05 | 6.33 | 6.26 | 6.49 | 6.24 | 0.07 | 0.39 |
| 24-h drip loss, % | 2.60 ^a | 1.98 ^{ab} | 1.87 ^{ab} | 1.49 ^b | 1.73 ^b | 0.12 | 0.03 |
| 48-h drip loss, % | 3.35 | 3.24 | 2.85 | 2.63 | 2.86 | 0.16 | 0.56 |
| Cooking loss, % | 21.46 | 21.00 | 19.65 | 15.02 | 20.40 | 1.19 | 0.48 |
| a* _{45 min} | 10.43 ^b | 11.62 ^{ab} | 13.27 ^a | 13.14 ^a | 13.01 ^a | 0.35 | 0.01 |
| b* _{45 min} | 8.80 | 8.96 | 8.74 | 9.39 | 8.61 | 0.12 | 0.35 |
| L* _{45 min} | 40.94 | 38.37 | 37.60 | 38.36 | 37.53 | 0.56 | 0.31 |
| a* _{24 h} | 12.04 | 13.22 | 12.88 | 12.52 | 12.19 | 0.39 | 0.90 |
| b* _{24 h} | 11.08 | 10.43 | 10.37 | 10.33 | 10.02 | 0.24 | 0.77 |
| L* _{24 h} | 43.44 | 40.42 | 41.37 | 40.28 | 40.26 | 0.56 | 0.35 |

^{a,b}Means within a row with different superscripts differ significantly at $P < 0.05$.

¹pH_{45 min} or pH_{24 h}, pH at 45 min or 24 h postmortem; a*_{45 min} or a*_{24 h}, redness at 45 min or 24 h postmortem; b*_{45 min} or b*_{24 h}, yellowness at 45 min or 24 h postmortem; L*_{45 min} or L*_{24 h}, lightness at 45 min or 24 h postmortem.

²Control, basal diet; CG, basal diet with 0.50% coarse *Ginkgo biloba* leaf powder; UG1, UG2, and UG3, basal diet with respectively 0.05%, 0.25% and 0.50% ultrafine *Ginkgo biloba* leaf powder.

³SEM, standard error of the mean based on pooled estimate of variation.

composition of both longissimus dorsi muscle and leg muscle in pigs were presented in Table V. Dietary treatments had no effect ($P > 0.05$) on the content of moisture, crude protein, ether extract or crude ash of neither longissimus dorsi muscle nor leg muscle, indicating that the deposition of these nutrients in muscles might not be affected by *G. biloba* leaf powder.

Antioxidant enzyme activities and lipid peroxidation levels

Table VI showed the antioxidant enzyme activities and lipid peroxidation of both longissimus dorsi muscle and leg muscle. The T-SOD activity of the longissimus dorsi muscle in UG2 group was higher ($P < 0.05$) than that of the CG and the control group, whereas dietary *G. biloba* leaf powder did not alter ($P > 0.05$) the T-AOC and GSH-Px activity of neither longissimus dorsi muscle nor leg muscle. SOD plays a vital role in balancing body

oxidation and antioxidation (Cao *et al.*, 2012). These results suggested that dietary addition of UG enhanced antioxidant activity of pig muscle by elevating the activity of SOD. Lipid peroxidation is a natural phenomenon involved in the loss of unsaturated lipids by peroxidation, thus, causes lipid degradation and membrane disruption in the muscle (Bai *et al.*, 2013). MDA is widely used as a biomarker for oxidative deterioration of lipid in muscle (Corino *et al.*, 1999; Liu *et al.*, 2009). In this trial, dietary addition of CG, UG1 and UG2 decreased ($P < 0.05$) the concentration of MDA in longissimus dorsi muscle compared with the control group. The MDA concentration of leg muscle was also decreased ($P < 0.05$) in UG groups. These results indicated that dietary addition of *G. biloba* leaf powder could alleviate lipid peroxidation in muscle. Some studies reported that flavonoids could inhibit the formation of

Table IV.- Meat quality of leg muscle in pigs fed diets supplemented with *G. biloba* leaf powder.

| Item ¹ | Treatments ² | | | | | SEM ³ | P-value |
|----------------------|-------------------------|-------------------|--------------------|-------------------|--------------------|------------------|---------|
| | Control | CG | UG1 | UG2 | UG3 | | |
| pH _{45 min} | 6.58 | 6.60 | 6.53 | 6.65 | 6.40 | 0.05 | 0.68 |
| pH _{24 h} | 5.79 | 5.79 | 5.84 | 5.86 | 5.73 | 0.03 | 0.71 |
| 24-h drip loss, % | 1.65 ^a | 1.23 ^b | 1.53 ^{ab} | 1.18 ^b | 1.50 ^{ab} | 0.06 | 0.04 |
| 48-h drip loss, % | 3.10 | 2.77 | 2.73 | 2.72 | 3.07 | 0.11 | 0.75 |
| Cooking loss, % | 28.97 | 27.66 | 25.09 | 23.31 | 28.69 | 1.02 | 0.36 |
| a* _{45 min} | 3.69 ^b | 4.36 ^a | 4.77 ^a | 4.48 ^a | 4.48 ^a | 0.14 | 0.04 |
| b* _{45 min} | 8.89 | 8.81 | 8.30 | 8.79 | 8.51 | 0.09 | 0.20 |
| L* _{45 min} | 42.03 | 40.42 | 40.09 | 41.11 | 40.21 | 0.29 | 0.19 |
| a* _{24 h} | 5.10 | 5.46 | 6.21 | 5.70 | 6.40 | 0.23 | 0.39 |
| b* _{24 h} | 10.38 | 10.91 | 10.44 | 10.13 | 10.37 | 0.20 | 0.86 |
| L* _{24 h} | 45.98 | 45.51 | 43.67 | 43.69 | 43.50 | 0.48 | 0.33 |

^{a,b}Means within a row with different superscripts differ significantly at P<0.05.

¹pH_{45 min} or pH_{24 h}, pH at 45 min or 24 h postmortem; a*_{45 min} or a*_{24 h}, redness at 45 min or 24 h postmortem; b*_{45 min} or b*_{24 h}, yellowness at 45 min or 24 h postmortem; L*_{45 min} or L*_{24 h}, lightness at 45 min or 24 h postmortem.

²Control, basal diet; CG, basal diet with 0.50% coarse *Ginkgo biloba* leaf powder; UG1, UG2, and UG3, basal diet with respectively 0.05%, 0.25% and 0.50% ultrafine *Ginkgo biloba* leaf powder.

³SEM, standard error of the mean based on pooled estimate of variation.

Table V.- Meat composition of longissimus dorsi muscle and leg muscle in pigs fed diets supplemented with *G. biloba* leaf powder (in living basis, %).

| Item | Treatments ¹ | | | | | SEM ² | P-value |
|---------------------------------|-------------------------|-------|-------|-------|-------|------------------|---------|
| | Control | CG | UG1 | UG2 | UG3 | | |
| Longissimus dorsi muscle | | | | | | | |
| Moisture | 71.27 | 71.78 | 70.61 | 71.95 | 71.96 | 0.25 | 0.42 |
| Crude protein | 20.81 | 20.68 | 20.48 | 21.17 | 21.11 | 0.20 | 0.84 |
| Ether extract | 4.14 | 4.12 | 4.37 | 4.47 | 4.24 | 0.09 | 0.80 |
| Crude ash | 1.16 | 1.16 | 1.23 | 1.15 | 1.13 | 0.01 | 0.33 |
| Leg muscle | | | | | | | |
| Moisture | 72.40 | 73.39 | 72.48 | 73.79 | 73.73 | 0.30 | 0.45 |
| Crude protein | 23.91 | 22.40 | 23.62 | 22.64 | 22.56 | 0.28 | 0.35 |
| Ether extract | 1.56 | 1.47 | 1.71 | 1.32 | 1.47 | 0.06 | 0.43 |
| Crude ash | 1.11 | 1.23 | 1.13 | 1.29 | 1.18 | 0.03 | 0.39 |

¹ Control, basal diet; CG, basal diet with 0.50% coarse *Ginkgo biloba* leaf powder; UG1, UG2, and UG3, basal diet with respectively 0.05%, 0.25% and 0.50% ultrafine *Ginkgo biloba* leaf powder.

² SEM, standard error of the mean based on pooled estimate of variation

oxygen radicals (superoxide ions and hydroxyl radicals) and scavenge superoxide radicals which are strong peroxidative agents (Facino *et al.*, 1990; Jia *et al.*, 1999), and terpenoids could protect rat heart through inhibiting the formation of free radical (Pietri *et al.*, 1997). These support our proposition that antioxidant improvement in muscle might be due to the antioxidant capacity of flavonoids and terpenoids in *G. biloba* leaf. In our study, UG inclusion increased the activity of T-SOD in longissimus dorsi muscle and decreased the content of MDA in leg muscle, whereas CG inclusion exerted no

significant effect on this compared with the control group, implying that pigs in UG groups had better ability in enhancing body antioxidant status than those in CG group. These results accorded with the studies of Zhang *et al.* (2009), who observed that dietary addition of ginger powder ranging from 300 to 8.4 μm increased T-SOD activity and decreased serum MDA concentration in serum of broilers. Similar results were found by Wu *et al.* (2014), who demonstrated that fine but not coarse oolong tea (*Camellia sinensis*) powder significantly increased SOD activity and decreased MDA content in serum

Table VI.- Antioxidant enzyme activities and lipid peroxidation levels in longissimus dorsi muscle and leg muscle of pigs fed diets supplemented with *G. biloba* leaf powder.

| Item ¹ | Treatments ² | | | | | SEM ³ | P-value |
|---------------------------------|-------------------------|--------------------|---------------------|--------------------|---------------------|------------------|---------|
| | Control | CG | UG1 | UG2 | UG3 | | |
| Longissimus dorsi muscle | | | | | | | |
| T-SOD, U/mg prot | 31.35 ^b | 32.31 ^b | 35.30 ^{ab} | 37.23 ^a | 33.76 ^{ab} | 0.72 | 0.03 |
| GSH-Px, U/mg prot | 6.67 | 7.34 | 7.30 | 8.29 | 8.60 | 0.33 | 0.38 |
| T-AOC, U/mg prot | 0.19 | 0.23 | 0.20 | 0.20 | 0.23 | 0.01 | 0.73 |
| MDA, nmol/mg prot | 0.78 ^a | 0.67 ^b | 0.67 ^b | 0.65 ^b | 0.70 ^{ab} | 0.02 | 0.04 |
| Leg muscle | | | | | | | |
| T-SOD, U/mg prot | 26.14 | 25.10 | 26.98 | 26.64 | 27.57 | 0.67 | 0.86 |
| GSH-Px, U/mg prot | 2.21 | 2.23 | 2.61 | 2.56 | 2.90 | 0.23 | 0.91 |
| T-AOC, U/mg prot | 0.06 | 0.06 | 0.07 | 0.07 | 0.06 | 0.01 | 0.97 |
| MDA, nmol/mg prot | 1.06 ^a | 0.96 ^{ab} | 0.93 ^b | 0.91 ^b | 0.94 ^b | 0.02 | 0.04 |

^{a,b} Means within a row with different superscripts differ significantly at P<0.05.

¹ T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

² Control, basal diet; CG, basal diet with 0.50% coarse *Ginkgo biloba* leaf powder; UG1, UG2, and UG3, basal diet with respectively 0.05%, 0.25% and 0.50% ultrafine *Ginkgo biloba* leaf powder.

³ SEM, standard error of the mean based on pooled estimate of variation.

compared with the control group. As it is known that flavonoids and terpenoids are the active components in *G. biloba* leaf influencing its antioxidant activity, both of which are present in leaf cells, coarse grinding may not be able to thoroughly break up the cell walls (Zhao *et al.*, 2009) of *G. biloba* leaf and thus active components may be difficult to release.

CONCLUSION

In conclusion, dietary supplementation of *G. biloba* leaf powder had no significant effect on neither growth performance nor meat chemical composition in growing-finishing pigs. Addition of *G. biloba* leaf powder could improve meat quality and enhance antioxidant capacity of muscle and this effect was more pronounced when supplementing ultrafine *G. biloba* leaf powder.

ACKNOWLEDGEMENTS

This study was supported by the Science and Technology Development Plan of Jiangsu province in China (BN2012043). The technical assistance of our labmates are gratefully acknowledged. All authors have approved the submission of this manuscript and declared no conflict of interest.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

AOAC, 1990. *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.

- Bai, H.X., Chang, Q.F., Shi, B.M. and Shan, A.S., 2013. Effects of fulvic acid on growth performance and meat quality in growing-finishing pigs. *Livest. Sci.*, **158**: 118-123.
- Cao, F.L., Zhang, X.H., Yu, W.W., Zhao, L.G. and Wang, T., 2012. Effect of feeding fermented *Ginkgo biloba* leaves on growth performance, meat quality, and lipid metabolism in broilers. *Poult. Sci.*, **91**: 1210-1221.
- Cordeiro, G.C., Toledo Filho, R.D., Tavares, L.M. and Fairbairn, E.D.M.R., 2009. Ultrafine grinding of sugar cane bagasse ash for application as pozzolanic admixture in concrete. *Cem. Concr. Res.*, **39**: 110-115.
- Corino, C., Oriani, G., Pantaleo, L., Pastorelli, G. and Salvatori, G., 1999. Influence of dietary vitamin E supplementation on "heavy" pig carcass characteristics, meat quality, and vitamin E status. *J. Anim. Sci.*, **77**: 1755-1761.
- Fastinger, N.D. and Mahan, D.C., 2003. Effect of soybean meal particle size on amino acid and energy digestibility in grower-finisher swine. *J. Anim. Sci.*, **81**: 697-704.
- Facino, R.M., Carini, M., Franzoi, L., Pirola, O. and Bosisio, E., 1990. Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (compositae). *Pharmacol. Res.*, **22**: 709-721.
- Franco, D., Bispo, E., González, L., Vázquez, J.A. and Moreno, T., 2009. Effect of finishing and ageing time on quality attributes of loin from the meat of Holstein-Friesian cull cows. *Meat Sci.*, **83**: 484-491.
- Franco, D., Vazquez, J.A. and Lorenzo, J.M., 2014. Growth performance, carcass and meat quality of the Celta pig crossbred with Duroc and Landrace genotypes. *Meat Sci.*, **96**: 195-202.
- Goh, L.M., Barlow, P.J. and Yong, C.S., 2003. Examination of antioxidant activity of *Ginkgo biloba* leaf infusions. *Fd. Chem.*, **82**: 275-282.

- Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G., 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J. Nutr.*, **104**: 580-587.
- Huff-Lonergan, E., Baas, T.J., Malek, M., Dekkers, J.C., Prusa, K. and Rothschild, M.F., 2002. Correlations among selected pork quality traits. *J. Anim. Sci.*, **80**: 617-627.
- Jacobs, B.P. and Browner, W.S., 2000. *Ginkgo biloba*: a living fossil. *The Am. J. Med.*, **108**: 341-342.
- Jia, Z.S., Tang, M.C. and Wu, J.M., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Fd. Chem.*, **64**: 555-559.
- Kobus-Cisowska, J., Flaczyk, E., Rudzińska, M. and Kmiecik, D., 2014. Antioxidant properties of extracts from *Ginkgo biloba* leaves in meatballs. *Meat Sci.*, **97**: 174-180.
- Lee, J.H., Kim, Y.G., Ryu, S.Y., Cho, M.H. and Lee, J., 2014. Ginkgolic acids and *Ginkgo biloba* extract inhibit Escherichia coli O157: H7 and Staphylococcus aureus biofilm formation. *Int. J. Fd. Microbiol.*, **174**: 47-55.
- Li, H., Li, F., Yang, F., Fang, Y., Xin, Z., Zhao, L. and Hu, Q., 2008. Size effect of Se-enriched green tea particles on in vitro antioxidant and antitumor activities. *J. Agric. Fd. Chem.*, **56**: 4529-4533.
- Liu, H.W., Gai, F., Gasco, L., Brugiapaglia, A., Lussiana, C., Guo, K.J., Tong, J.M. and Zoccarato, I., 2009. Effects of chestnut tannins on carcass characteristics, meat quality, lipid oxidation and fatty acid composition of rabbits. *Meat Sci.*, **83**: 678-683.
- Lu, G., Wu, Y., Mak, Y.T., Wai, S.M., Feng, Z.T., Rudd, J.A. and Yew, D.T.W., 2006. Molecular evidence of the neuroprotective effect of *Ginkgo biloba* (EGb761) using bax/bcl-2 ratio after brain ischemia in senescence-accelerated mice, strain prone-8. *Brain Res.*, **1090**: 23-28.
- Ma, P.Y., Fu, Z.Y., Su, Y.L., Zhang, J.Y., Wang, W.M., Wang, H., Wang, Y.C. and Zhang, Q.J., 2009. Modification of physicochemical and medicinal characterization of Liuwei Dihuang particles by ultrafine grinding. *Powder Technol.*, **191**: 194-199.
- Macit, M., Aksakal, V., Emsen, E., Aksu, M.I., Karaoglu, M. and Esenbuga, N., 2003. Effects of vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. *Meat Sci.*, **63**: 51-55.
- Oyanagui, Y., 1984. Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Biochem.*, **142**: 290-296.
- Pietri, S., Maurelli, E., Drieu, K. and Culcasi, M., 1997. Cardioprotective and anti-oxidant effects of the terpenoid constituents of *Ginkgo biloba* extract (EGb 761). *J. Mol. Cell. Cardiol.*, **29**: 733-742.
- Placer, Z.A., Cushman, L.L. and Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem.*, **16**: 359-364.
- Puebla-Pérez, A.M., Lozoya, X. and Villaseñor-García, M.M., 2003. Effect of *Ginkgo biloba* extract, EGb 761, on the cellular immune response in a hypothalamic-pituitary-adrenal axis activation model in the rat. *Int. Immunopharmacol.*, **3**: 75-80.
- Rong, Y., Geng, Z. and Lau, B.H., 1996. *Ginkgo biloba* attenuates oxidative stress in macrophages and endothelial cells. *Free Radical Biol. Med.*, **20**: 121-127.
- Schilling, M.W., Radhakrishnan, V., Thaxton, Y.V., Christensen, K., Thaxton, J.P. and Jackson, V., 2008. The effects of broiler catching method on breast meat quality. *Meat Sci.*, **79**: 163-171.
- Şener, G., Sehirlı, O., Tozan, A., Velioğlu-Ovunç, A., Gedik, N. and Omurtag, G.Z., 2007. *Ginkgo biloba* extract protects against mercury (II)-induced oxidative tissue damage in rats. *Fd. Chem. Toxicol.*, **45**: 543-550.
- Tkacova, K. and Stevulova, N., 1998. Selected problems of the dispersity analysis of milled ultrafine powders. *Freib. Forsch. A (Partik.) A*, **841**: 14-25.
- Van Beek, T.A. and Montoro, P., 2009. Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. *J. Chromatogr. A*, **1216**: 2002-2032.
- Wu, P., Wen, C., Leng, Z.X. and Zhou, Y.M., 2014. Effect of oolong tea powder particle size on growth performance, fat deposition, meat quality and antioxidant capacity in meat ducks. *Anim. Feed Sci. Tech.*, **194**: 131-135.
- Yao, P., Li, K., Jin, Y., Song, F., Zhou, S., Sun, X., Andreas, K., Liu, L. and Liu, L., 2006. Oxidative damage after chronic ethanol intake in rat tissues: Prophylaxis of *Ginkgo biloba* extract. *Fd. Chem.*, **99**: 305-314.
- Yu, W.W., Zhang, X.H., Ahmad, H., Zhao, L.G., Wang, T. and Cao, F.L., 2015. Intestinal absorption function of broiler chicks supplemented with *Ginkgo* leaves fermented with *Bacillus* species. *Pakistan J. Zool.*, **47**: 479-490.
- Zhang, G.F., Yang, Z.B., Wang, Y., Yang, W.R., Jiang, S.Z. and Gai, G.S., 2009. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poult. Sci.*, **88**: 2159-2166.
- Zhang, X.H., Cao, F.L., Sun, Z.Y., Yu, W.W., Zhao, L.G., Wang, G.B. and Wang, T., 2012. Effect of feeding *Aspergillus niger*-fermented *Ginkgo biloba* leaves on growth, small intestinal structure and function of broiler chicks. *Livest. Sci.*, **147**: 170-180.
- Zhang, X.L., Wu, D.W., Lu, P., Xue, W.Y., Yuan, Z.H., Wang, G.Q. and Zhou, Y.M., 2014. Effects of ordinary and ultrafine grinding on the dissolution of total flavonoids and terpenoids for *Ginkgo* leaves (in Chinese). *Cereal Feed Ind.*, **6**: 45-47.
- Zhao, X., Yang, Z., Gai, G. and Yang, Y., 2009. Effect of superfine grinding on properties of ginger powder. *J. Fd. Eng.*, **91**: 217-222.
- Zhu, K., Huang, S., Peng, W., Qian, H. and Zhou, H., 2010. Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. *Fd. Res. Int.*, **43**: 943-948.

