Effect of dietary supplementation with sugar cane extract on meat quality and oxidative stability in finishing pigs

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Abstract
The aim of the present study was to investigate the effect of dietary supplementation with sugar cane extract (SCE) on meat quality and oxidative stability of Longissimus dorsi muscle in finishing pigs. Eighteen barrows (Duroc × Landrace × Jiaxing Black), with an average initial body weight of 62.1 ± 5.0 kg, were randomly allotted to 1 of 3 diets with 6 replicates per treatment for 42 days. The diets comprised a normal diet and the normal diets supplemented with 5 and 25 g/kg SCE. The results showed that SCE supplementation did not affect final body weight of finishing pigs. Dietary SCE supplementation significantly increased (P < 0.05) Longissimus dorsi muscle pH 24 h, and tended to reduce (P < 0.1) shear force, drip loss, myoglobin content, and peroxide and peroxy radicals. Dietary SCE treatments significantly increased (P < 0.05) malonaldehyde content and total superoxide dismutase activity in Longissimus dorsi muscle, and tended to reduce (P < 0.1) malonaldehyde content in serum. Altogether, these data indicate that SCE is an effective feed additive to improve pork meat quality, and the underlying mechanism may be partly due to the improved oxidative stability induced by dietary SCE supplementation.

Keywords:
Sugar cane extract
Feed additive
Meat quality
Oxidative stability
Finishing pigs

1. Introduction

With the development of meat industry, considerable attention has been paid to the improvement of meat quality parameters. Meat producers consistently produce safe, healthy and tasty meat for consumers, accompanied by eliminating deteriorative phenomenon that negatively affects meat quality. As a major cause of meat deterioration (Asghar et al., 1988), lipid oxidation can produce toxic compounds, such as fatty acid peroxides, cholesterol hydroperoxide and peroxy radicals (Grün et al., 2006) that adversely influence muscle oxidative stability. What is more, muscle oxidative stability is related to many aspects of meat quality that are represented by postmortem pH, color, water holding capacity, etc. It is generally believed that lipid oxidation can be inhibited by synthetic and natural antioxidants. However, the resistance to food additives of synthetic substances has increased due to safety and health concern (Karre et al., 2013). Therefore, there is now considerable appreciation of enhancing pork quality through antioxidant properties of natural occurring substances, such as natural plant extract (Rossi et al., 2013).

The sugar cane (Saccharum officinarum L.), one of the major sources of sugar, is a widely cultivated plant throughout the whole world. Sugar cane and its derived products have displayed a wide range of biological activities, including antioxidant, anti-inflammatory, antiatherosclerotic, immune-stimulation, DNA damage protecting activity (El-Abasy et al., 2002; Chung et al., 2011). Sugar cane extract (SCE), a natural byproduct in sugar cane industry, has been found the antioxidant property that is due to the presence of high content of phenolic compounds, primarily, sinapic acid, chlorogenic acid, apigenin derivatives, and tricin derivatives in sugar cane juice (Duarte-Almeida et al., 2006). These natural...
bioactive compounds, present in vegetables, beans, fruits, are generally regarded as safe chemicals exhibiting low toxicity. To the best of our knowledge, although phenolic entities in sugar cane could act as antioxidants, no relevant study was observed for effects of SCE on meat quality. On this basis, we hypothesized that dietary supplementation with SCE may change meat quality characteristics through influencing antioxidative status in finishing pigs.

The aim of the present study is to evaluate the effect of dietary SCE supplementation on meat quality parameters and oxidative stability of *Longissimus dorsi* (LD) muscle in Duroc × Landrace × Jiaxing Black crossbred pigs.

2. Materials and methods

2.1. Material

Sugar cane extract was produced and kindly provided by Shin Mitsui Sugar Co., Ltd. (Tokyo, Japan). The original material for SCE production was the sugar cane juice from the raw sugar manufacturing process. After removing most of the sugar components including glucose, fructose and sucrose from sugar cane juice, the residue was dried and adsorbed to bread crumb to produce SCE, according to the production manual. The nutrient content and phenolic content of SCE are presented in Table 1.

2.2. Animals and experimental design

All procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University. Eighteen crossbred pigs (Duroc × Landrace × Jiaxing Black) with an average initial body weight of 62.1 ± 5.0 kg (means ± SD) were randomly divided into 3 groups with 6 replicates of 1 each as follows: a control group was fed a normal diet (basal diet, Table 2) and the other 2 groups were fed the normal diet supplemented with 5 and 25 g/kg SCE dietary for 42 days. Pigs were housed in individual pens and allowed *ad libitum* access to feed and water.

2.3. Slaughter and sampling

At the completion of the feeding period, all pigs were subjected to feed deprivation for 12 h but free drinking including the time during transportation and then slaughtered humanely by bleeding directly. Each sample was determined 3 times in different locations and the average value was obtained.

The pork drip loss was measured as below. Briefly, a muscle section (size 2 cm × 3 cm × 5 cm) was manually trimmed and weighed at about 45 min postmortem, followed by suspending from an iron wire hook within an inflated and sealed plastic bag at 0 to 4 °C for 24 h. After that, the sample was taken out from the plastic, wiped dry on filter paper and reweighed. Drip loss was expressed as follows: Drip loss (%) = [(Initial weight – Final weight)/Initial weight] × 100.

Table 2

| Ingredients, % | Content                | Nutrients, %  | Content |
|---------------|------------------------|---------------|---------|
| Corn          | 77                     | Digestible energy, MJ/kg | 13.90   |
| Soybean meal  | 18                     | Crude protein  | 14.81   |
| Wheat bran    | 2                      | Calcium       | 0.64    |
| Dicalcium phosphate | 0.9                  | Total phosphorus| 0.50    |
| Limestone     | 1                      | Available phosphorus| 0.29    |
| Lysine HCl    | 0.2                    | Lysine        | 0.82    |
| NaCl          | 0.3                    | Methionine    | 0.25    |
| Premix1       | 0.6                    | Threonine     | 0.55    |
| Total         | 100                    | Tryptophan    | 0.16    |

1. Provided the following per kg diet for finishing pigs: 100 mg of Fe (as ferrous sulfate); 15 mg of Cu (as copper sulfate); 120 mg of Zn (as zinc sulfate); 40 mg of Mn (as manganese sulfate); 0.3 mg of Se (as Na2SeO3); 0.25 mg of I (as KI); 13,500 IU of vitamin A; 2,250 IU of vitamin D3; 24 IU of vitamin E; 6.2 mg of riboflavin; 25 mg of nicotinic acid; 15 mg of pantothenic acid; 1.2 mg of vitamin B12; 0.15 mg of biotin.

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The meat color L* (lightness), a* (redness) and b* (yellowness) were measured at approximately 24 h postmortem using a Colorimeter (CR-10, Konica Minolta, Japan). Measurements were made at 3 different areas of the samples and the average values of L*, a* and b* were recorded.

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Table 1

| Nutrient content, % | Content                  | Phenolic compounds1 | Content       |
|---------------------|--------------------------|--------------------|---------------|
| Moisture            | 5.63                     | Total phenolic content | 89.39         |
| Crude protein       | 13.65                    | Sinapic acid       | 1.16          |
| Crude fat           | 2.99                     | Chlorogenic acid   | 10.70         |
| Crude fiber         | 1.32                     | Apigenin           | 5.68          |
| Ash                 | 16.64                    | Tricin             | 7.31          |
| Nitrogen free extracts | 59.77                   | Gallic acid        | 8.12          |

1. Total phenolic content is expressed as mg/g SCE dry matter. Sinapic acid, chlorogenic acid, apigenin, tricin and gallic acid are expressed as mg/100 g SCE dry matter.
2.6. Myofiber cross sectional area (CSA) determination

The sample myofiber CSA was determined by histomorphology observation, according to Zhang et al. (2015) and modified as below. Briefly, transverse serial sections of 5 μm were cut from embedded muscle samples on a Leica RM2235 rotary cutting machine (Leica Instrument GmbH, Germany) followed by HE staining. Three fascicles of one sample were randomly selected from different field of microscope (Nikon YS100, Japan). Then myofibers cross sectional image was captured at a magnification of 40, and the myofiber CSA was measured using Image-Pro software (Image-Pro Plus 6.0, Silver Spring, MD, USA).

2.7. Enzyme activity and malondialdehyde (MDA) content measurement

About 150 mg frozen muscle sample was homogenized on ice in 1.35 mL of 0.9% saline and then centrifuged at 800 × g for 10 min at 4 °C. The supernatant, 10% muscle homogenate, was used to measure MDA content, lactate dehydrogenase (LDH), glutathione peroxidase (GPx), total superoxide dismutase (T-SOD) and catalase (CAT) in triplicate at appropriate dilutions. The serum was also analyzed for MDA content, GPx, T-SOD and CAT. The assays were conducted with the commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

2.8. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test using Prism software (GraphPad Prism Software, San Diego, CA, USA). Results were expressed as means ± standard error of the mean (SEM). Differences with \( P < 0.05 \) were considered statistically significant, whereas a \( P \)-value between 0.05 and 0.10 was classified as a trend.

3. Results

3.1. Meat quality

Pigs exhibited (Table 3) similar final body weight among groups after 42 days of feeding (\( P > 0.05 \)). No effect of dietary SCE supplementation was detected on \( pH_{24} \) min, L’, a’, b’ and cooking loss \( (P > 0.05) \). However, dietary SCE treatments significantly increased \( (P < 0.05) \) LD muscle \( pH_{24} \), while tended to decrease \( (P < 0.1) \) and significantly reduced \( (P < 0.05) \) shear force and drip loss at 5 and 25 g/kg, respectively. The measured meat quality traits were not significantly different between the 5 and 25 g/kg SCE-supplemented groups \( (P > 0.05) \).

### Table 3

| Item | Control | 5 g/kg SCE | 25 g/kg SCE |
|------|---------|------------|-------------|
| Initial body weight, kg | 61.92 ± 2.22 | 61.83 ± 1.60 | 62.42 ± 2.59 |
| Final body weight, kg | 94.56 ± 3.67 | 97.71 ± 1.97 | 94.06 ± 2.93 |
| \( pH_{45} \) min | 6.16 ± 0.06 | 6.14 ± 0.12 | 6.13 ± 0.15 |
| \( pH_{24} \) | 5.75 ± 0.05* | 6.05 ± 0.05* | 5.96 ± 0.05b |
| L’ | 43.11 ± 0.95 | 42.81 ± 0.87 | 43.19 ± 0.38 |
| a’ | 8.30 ± 0.35 | 8.38 ± 0.79 | 8.66 ± 0.31 |
| b’ | 11.62 ± 0.78 | 13.19 ± 0.77 | 13.25 ± 0.27 |
| Shear force, kg | 5.03 ± 0.29b | 4.08 ± 0.19b | 3.65 ± 0.32a |
| Drip loss, % | 6.62 ± 0.65b | 5.11 ± 0.25b | 4.72 ± 0.18b |
| Cooking loss, % | 21.03 ± 1.73 | 21.85 ± 0.54 | 21.83 ± 1.55 |

L’ = lightness, a’ = redness, b’ = yellowness. No effect of dietary SCE supplementation was detected (Table 4) on moisture, crude protein, IMF and ash content \( (P > 0.05) \). However, LD muscle myofiber CSA was decreased (Table 5) by the supplementation of SCE when compared with the control group \( (P < 0.05) \). Dietary SCE treatments tended to reduce \( (P < 0.1) \) and significantly decreased \( (P < 0.05) \) LDH activity at 5 and 25 g/kg, respectively. Measured muscle chemical composition (Table 4), myofiber CSA and LDH activity (Table 5) were not significantly different between the 5 and 25 g/kg SCE-supplemented groups \( (P > 0.05) \).

### Table 4

| Item | Control | 5 g/kg SCE | 25 g/kg SCE |
|------|---------|------------|-------------|
| Moisture | 73.82 ± 0.36 | 73.60 ± 0.32 | 73.91 ± 1.13 |
| IMF | 2.70 ± 0.11 | 2.96 ± 0.17 | 3.04 ± 0.29 |
| Crude protein | 20.68 ± 0.12 | 20.21 ± 0.14 | 20.12 ± 0.46 |
| Ash | 1.92 ± 0.08 | 1.77 ± 0.07 | 1.83 ± 0.04 |

IMF = intramuscular fat content. No effect of dietary SCE supplementation was detected (Table 4) on muscle moisture, crude protein, IMF and ash content \( (P > 0.05) \). However, LD muscle myofiber CSA was decreased (Table 5) by the supplementation of SCE when compared with the control group \( (P < 0.05) \). Dietary SCE treatments tended to reduce \( (P < 0.1) \) and significantly decreased \( (P < 0.05) \) LDH activity at 5 and 25 g/kg, respectively. Measured muscle chemical composition (Table 4), myofiber CSA and LDH activity (Table 5) were not significantly different between the 5 and 25 g/kg SCE-supplemented groups \( (P > 0.05) \).

3.2. Muscle chemical composition, myofiber cross sectional area and lactate dehydrogenase activity

Dietary SCE supplementation did not affect \( (P > 0.05) \) muscle GPx and CAT activity but resulted in decreased \( (P < 0.05) \) muscle T-SOD activity and MDA content (Table 6). Dietary SCE treatments did not affect \( (P > 0.05) \) measured serum antioxidative enzyme activities but tended to reduce \( (P < 0.1) \) serum MDA content (Table 7). There were no significant differences in measured antioxidative enzyme activities and MDA content of muscle (Table 6) and serum (Table 7) between 5 and 25 g/kg SCE supplementation groups \( (P > 0.05) \).

4. Discussion

Meat quality traits can be defined by physicochemical properties, mainly color, pH, water-holding capacity (WHC), tenderness, nutrient composition, etc. It is meaningful to improve these meat quality traits, because they are closely correlated to consumer acceptance and thereby financial implications. This study is the first to demonstrate that dietary supplementation with SCE increased \( pH_{24} \) of LD muscle of finishing pigs, while decreased shear force, drip loss and cooking loss. Therefore, SCE seems to function as a helpful feed additive to improve pork meat quality.

Meat quality development is often influenced by post-slaughter muscle metabolism, which is indicated by the rate of lactic acid generation and subsequent reduction of pH (Park et al., 2010). Lactate dehydrogenase is a key enzyme in the conversion of pyroracemic acid to lactic acid under postmortem anaerobic conditions in the muscle (Li et al., 2015). Previous studies have reported that LDH activity was positively and negatively correlated with lactic acid and ultimate pH in muscle, respectively (Pérez et al., 2002; Marrocco et al., 2011). The abnormally low pH induces denaturation of sarcoplasmic and myofibrillar proteins and damages integrity of muscle cell membrane, resulting in worse WHC and unattractive appearance (Zhang et al., 2014). Studies have
reported that low ultimate pH was usually accompanied by low WHC (Allison et al., 2003; HUFFLONER AND LONGER, 2005), and a lower WHC has been observed with an enlargement of myofiber CSA (DiETL et al., 1993). Moreover, increased ultimate pH has a beneficial influence on the tenderness of meat (HUFFLONER et al., 2000). RYU and KIM (2005) have also reported that there is an inverse correlation between myofiber CSA and tenderness. In addition, a higher body weight was indicated to be accompanied by an enlarged myofiber size (KAMASWAMY et al., 1992), which suggests a negative correlation between body weight and tenderness of muscle. In the present study, dietary SCE supplementation did not affect final body of finishing pigs, while decreased muscle LDH activity and myofiber CSA. Therefore, the reduced LDH activity and myofiber size may be the partial explanation for the improved meat quality traits in response to SCE treatment.

Under the physiological status, the molecular oxygen undergoes several types of biochemical reactions that leads to the formation of free radicals. Free radicals are potentially harmful because of their high biochemical reactivity and cause the oxidation of biomolecules, for example protein, lipid, and nucleic acid, which results in cell injury or even death (Fang et al., 2002). It was reported that lipid peroxidation led to disruption of muscle cell membrane integrity, which may badly induce exudative loss from meat (Buckley et al., 1995). Malondialdehyde is the main end-products of lipid peroxides that generally used as an indicator of an increased oxidative stress in the body (Gawe et al., 2004), and is considered to be a carcinogenic initiator and mutagen, which can endanger the safety of food (Fernandez et al., 1997). In addition, meat tenderization process can be interfered by the suppressed calpain activity and slowed rate of proteolysis occurring in oxidative conditions, which increases meat toughness ( Rowe et al., 2004). Therefore, oxidation in meat may involve in the decline in meat quality characteristics and contribute to potential health risks.

Antioxidants have the capacity to avoid tissue damage by preventing the formation of radicals, by scavenging them or by promoting their decomposition (Falowo et al., 2014). The principal defense systems which combat against free radicals inside body are enzymatic antioxidants including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and CAT, and non-enzymatic antioxidant nutrients (Fang et al., 2002). In recent years, natural plant extracts contain phenolic compounds that have been considered as potential dietary antioxidants to decrease lipid peroxidation in the body. For example, soybean isoflavone in broiler diets, and resveratrol (Zhang et al., 2015) and verbascoside (Rossi et al., 2015) in pig diets could improve meat quality by reducing lipid peroxidation and ameliorating antioxidative status. Previous studies have demonstrated that SCE had remarkable superoxide anion scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and FFKB12-Rapamycin-associated protein (FRAP) activity in vitro, and the high antioxidant activity was positively related to a high content of phenolic compounds existed in SCE (Nagai and Koge, 2000; Chung et al., 2011; Feng et al., 2014). In this study, SCE presented a high content of total phenolic compounds, among which, sinapic acid, chlorogenic acid, apigenin, tricin and gallic acid were detected. These phenolics detected are very well known for their antioxidant properties in different assay models (NičiFOROVIĆ and Abramovic, 2014; Sato et al., 2011; Sadasivam and Kumaresan, 2011; Duarte-Almeida et al., 2007) and hence, may play important roles in the antioxidant function of SCE. However, it is still unknown whether SCE could contribute to antioxidation like other plant extracts in vivo. Exquisitely, this study provides the first evidence that the dietary SCE supplementation reduced the MDA content of muscle and serum in finishing pigs which may indicate a decreased lipid peroxidation, and indirectly indicate an increased antioxidative capacity. Moreover, at the same point, decreased total SOD activity in muscle was also observed as the result of dietary SCE supplementation. Superoxide dismutase was an enzyme that converts superoxide radicals into hydrogen peroxide and oxygen, playing a crucial role in antioxidant defense (Thirach et al., 2007). Actually, SCE presents high superoxide anion scavenging activity same as SOD, indicating the scavenging activity turned into enzymatic activity (Nagai and Koge, 2000). KINOH et al. (2007) reported that oral administration of antioxidant containing plant-based SOD had the effect of lowering the activity and content of SOD inside body. Similarly, it seems likely that SCE may act as an exogenous SOD to eliminate free radicals and improve antioxidative statuses, and thereby suppress the generation of endogenous SOD, which results in lower SOD activity in muscle. Accordingly, it is appropriate to assume that antioxidant capacity is enhanced by the SCE treatment. In view of the going, we speculate that the improved pork quality in dietary SCE treatment of this study possibly ascribed to improved oxidative stability.

5. Conclusion

To our best knowledge, this study is the first to demonstrate that SCE can serve as an effective feed additive that beneficially improve

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**Table 5**

Effect of dietary sugar cane extract (SCE) supplementation on myofiber cross-sectional area (myo CSA) and lactate dehydrogenase (LDH) activities in Longissimus dorsi muscle of finishing pigs.  

| Item                  | Control  | 5 g/kg SCE | 25 g/kg SCE |
|-----------------------|----------|------------|-------------|
| Myofiber CSA, μm²     | 3.738 ± 0.247 | 3.786 ± 0.130<sup>a</sup> | 2.736 ± 0.396<sup>b</sup> |
| LDH, U/g protein      | 79.32 ± 7.58<sup>c</sup> | 62.59 ± 2.10<sup>d</sup> | 54.41 ± 4.20<sup>e</sup> |

<sup>a,b</sup> Within a row, means with different superscript letters are significantly different (P < 0.05).

<sup>1</sup> Data represent means ± SEM of 6 replicates.

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**Table 6**

Effect of dietary sugar cane extract (SCE) supplementation on Longissimus dorsi muscle MDA content and antioxidative enzyme activities of finishing pigs.  

| Item                  | Control  | 5 g/kg SCE | 25 g/kg SCE |
|-----------------------|----------|------------|-------------|
| MDA, nmol/mg protein  | 0.30 ± 0.01<sup>b</sup> | 0.22 ± 0.02<sup>a</sup> | 0.20 ± 0.03<sup>a</sup> |
| T-SOD, U/mg protein   | 48.72 ± 2.15<sup>b</sup> | 39.74 ± 3.43<sup>a</sup> | 38.24 ± 0.88<sup>a</sup> |
| CAT, U/mg protein     | 93.45 ± 5.01 | 94.26 ± 4.39 | 92.72 ± 4.14 |
| GPx, U/mg protein     | 26.73 ± 7.79 | 23.93 ± 5.00 | 26.06 ± 3.14 |

MDA — malonaldehyde; T-SOD — total superoxide dismutase; CAT — catalase; GPx — glutathione peroxidase.

<sup>a,b</sup> Within a row, means with different superscript letters are significantly different (P < 0.05).

<sup>1</sup> Data represent means ± SEM of 6 replicates.

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**Table 7**

Effect of dietary sugar cane extract (SCE) supplementation on serum MDA content and antioxidative enzyme activities of finishing pigs.  

| Item                  | Control  | 5 g/kg SCE | 25 g/kg SCE |
|-----------------------|----------|------------|-------------|
| MDA, nmol/mL          | 3.48 ± 0.17 | 3.04 ± 0.11 | 3.00 ± 0.16 |
| T-SOD, U/L            | 59.81 ± 1.72 | 50.25 ± 1.51 | 51.88 ± 2.57 |
| CAT, U/L              | 7.21 ± 0.86 | 7.45 ± 0.98 | 7.76 ± 0.72 |
| GPx, U/L              | 402.4 ± 24.78 | 402.6 ± 17.57 | 425.8 ± 18.78 |

MDA — malonaldehyde; T-SOD — total superoxide dismutase; CAT — catalase; GPx — glutathione peroxidase.

<sup>a,b</sup> Within a row, means with different superscript letters are significantly different (P < 0.05).

<sup>1</sup> Data represent means ± SEM of 6 replicates.
meat quality of finishing pigs. Furthermore, this study also provides the first evidence that dietary SCE supplementation can decrease myofiber CSA and LDH activity, and improve oxidative stability in pork, which may serve as useful information for understanding the underlying mechanisms of improved meat quality.

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