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## Effects of catechins on litter size, reproductive performance and antioxidative status in gestating sows


 Zhiyong Fan <sup>a,\*</sup>, Yong Xiao <sup>a</sup>, Yonghui Chen <sup>a</sup>, Xin Wu <sup>b</sup>, Guanglei Zhang <sup>a</sup>, Qinhuang Wang <sup>a</sup>, Chunyan Xie <sup>b</sup>
<sup>a</sup> Engineering Research Center for Feed Safety and Efficient Utilization of Ministry of Education, Institute of Animal Nutrition, Hunan Agricultural University, Changsha 410128, China

<sup>b</sup> Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

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## ABSTRACT

This study was conducted to investigate the effects of catechins on reproductive performance, antioxidative capacity and immune function of gestating sows. A total of 60 cross-bred (Landrace × Large White) multiparous sows were blocked by body weight, parity and backfat and randomly allocated to 1 of 5 treatments: 0, 100, 200, 300, or 400 mg/kg catechins. Dietary treatments were imposed from mating to d 40 of gestation of sows. At farrowing, litter total born, born alive, dead, and normal-(healthy piglets,  $\geq 0.85$  kg) and low-birth weight piglets ( $< 0.85$  kg) were recorded. Within  $3.00 \pm 0.50$  days after farrowing litter size was standardized to  $8.00 \pm 1.50$  piglets within treatment. The piglets were weighed at birth (d 1) and weaning (d 28). Sows serum samples were obtained from blood samples collected on d 40 of gestation for analyses of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), hydrogen peroxide ( $H_2O_2$ ), nitric oxide synthetase (NOS) and nitrogen monoxide (NO). Our results showed that supplementation of catechins at levels of 200 or 300 mg/kg led to improvements in litter born alive ( $P < 0.01$ ) and piglet born healthy ( $P < 0.01$ ) and a decrease in stillborn ( $P < 0.05$ ) at farrowing when compared with the control. In comparison with the control, catechins at any supplemental levels all enhanced the serum SOD ( $P < 0.05$ ) and CAT ( $P < 0.01$ ) activities of sows at farrowing but no obvious differences in the serum GSH-Px and NOS activities were observed in this trial ( $P > 0.05$ ). Sows received 200 mg catechin per kg diets showed a reduction ( $P < 0.05$ ) of the serum MDA level at farrowing compared with all other treatments. Sows received all the levels of catechin showed a reduction ( $P < 0.05$ ) of serum  $H_2O_2$  level compared with sows received the control diet on both d 40 of gestation and farrowing. Our results demonstrated that the catechins may be a potential antioxidant to increase the reproductive performance and antioxidative capacity of sows when it was added into diets during the early gestation.

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## 1. Introduction

Rapid fetal development during the gestation led to a catabolic status of pregnant women or dams which is known to contribute to the production of excessive free radicals including superoxide and hydrogen peroxide and the induction of systemic oxidative stress (Herrera and Ortega-Senovilla, 2010; Kim et al., 2013). Increased oxidative stress was reported to be an important factor causing decreased availability of antioxidants during late gestation, which could impaired placenta and fetal growth (Prater et al., 2008) and trigger a disrupted antioxidant system that was involved in a variety of pregnancy complications such as preterm labor, fetal growth restriction, preeclampsia and miscarriage (Gupta et al., 2003;

\* Corresponding author.

E-mail address: [fzyong04@163.com](mailto:fzyong04@163.com) (Z. Fan).

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Sugino et al., 2007). This elevated oxidative stress during gestation and lactation was likely to influence not only the litter performance, but also the well-being and health status of sows including impaired milk production, reproductive performance, and longevity (Agarwal et al., 2003; Jabbour et al., 2009; Zhao et al., 2011, 2013). Therefore, much attention has been paid to how to reduce maternal oxidative stress levels and inflammatory responses of highly prolific sows in late gestation by feed antioxidant additives. In numerous previous studies, antioxidants such as vitamin E, vitamin C, carotenoids, and selenium (Lykkesfeldt and Svendsen, 2007), fish oil and olive oil (Shen et al., 2015) and soy isoflavones (Hu et al., 2015) were added into the diet during gestation period in order to compensate for the substantial loss of these feed antioxidant additives. Excessive reactive oxygen and radical were actually produced from placental and maternal metabolism during the early pregnancy of sows. Although oxidative stress in late gestation was more serious than that in early gestation with the course of pregnancy (Berchieri-Ronchi et al., 2011; Casanueva and Viteri et al., 2003; Myatt and Cui, 2004), indicating that early pregnancy may be a key phase for prevention of oxidative damage.

Catechins are members of the flavonoid family and belong to plant polyphenolic constituents (Uzun et al., 2010), which are not only existing in a high concentration within tea, but also present in many foods, such as apples, grapes, vine and their processed beverages (Tichopad et al., 2005; Suzuki et al., 2007). Previous studies showed that catechins has a certain degree of hydrophobicity and can capture the  $\text{OH}^{-1}$ , which protect the DNA from the oxidant damage (Yoshioka et al., 1996); And catechins also alleviated the damage by up-regulating the expression of genes of some antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) so that increase those enzyme's production and activity (Mkimura et al., 2002). Previous studies showed that catechins prevented metal ions from participating in peroxidase reactions by binding them and had the potential to scavenge reactive oxygen and nitrogen species, thus reducing their damage to lipid membranes, proteins, and nucleic acids in cell-free systems (Wiseman et al., 1997). These findings indicated that catechins prevented metal ions from participating in peroxidase reactions by binding them and had the potential to scavenge reactive oxygen and nitrogen species (Wiseman et al., 1997). It was reported that catechins administration significantly decreased malondialdehyde (MDA) level and noticeably increased activities of CAT, GSH-Px and SOD, suggesting that catechins provided effective protection from oxidative damages through their antioxidant properties (Tarek et al., 2012). Therefore, it is believed that catechins have a beneficial role on physiological functions and biotransformation of physiological processes involved in the detoxification activities and preventing oxidative damage as a result of their ability to scavenge reactive oxygen species such as hydroxyl radical and superoxide anion (Galati et al., 2002) and metal chelating (Pedrielli and Skibsted, 2002), thereby providing some protection from toxic metabolic actions of oxidative stress (Suhel et al., 2006).

Considering the antioxidant properties of catechins and oxidative stress for highly prolific sows in early pregnancy, the aim of this study was to determine the effect of catechins supplementation in diets fed to sows in early pregnancy on reproductive performance and antioxidative status of gestating sows.

## 2. Materials and methods

This experiment was conducted at the Zhenghong Swine Research Farm in Miluo District, Hunan Province, China. This study was performed in accordance with Chinese Animal Welfare Act guidelines and approved by the Animal Care and Use Committee of

the Institute of Subtropical Agriculture, the Chinese Academy of Sciences (Wu et al., 2012).

### 2.1. Animals and experimental design

A total sixty of multiparous lactating sows (Landrace × Large White) were used in this experiment. The sows were evenly allocated by BW, back fat, expected farrowing date and parity into 5 dietary treatments, with 12 replicates. Litter size was standardized to  $8.00 \pm 1.50$  piglets within  $3.00 \pm 0.50$  days after farrowing. The sows were housed individually in crate stalls from mating. Five days before the expected date of farrowing/parturition, all the sows were moved into the individual farrowing crates with a heated piglet nest on d 109 of pregnancy. Rooms were ventilated mechanically.

The sows receive a gestational diet (Table 1) with levels of nutrients and minerals based on NRC (1998) recommendations from mating to d 40 of gestation, which is only different in the dose of catechins: 0 (Group I), 100 (Group II), 200 (Group III), 300 (Group IV) or 400 (Group V) mg catechins per kg diet. The sows were given 1.8, 2.3, and 2.5 kg/d during the pregnancy period, respectively, and thin sows were provided an extra amount of feed (0.3 kg/d). The day of farrowing sows did not receive any feed and the daily amount of feed was increased by 0.75 kg/d until ad libitum at d 4 of lactation, and thereafter the sows were fed twice daily, in the morning and evening, and had free access to water from nipple drinkers. The lactation length was 28 days. The litter total born, born alive, stillborn, and low-birth weight piglets (birth weight  $\leq 0.8$  kg) were recorded. Pigs born healthy number was obtained by the difference between the litter total born and stillborn and mummies. The piglets were weighed at farrowing. No creep feed was provided. Natural catechins (99% purity) were obtained from National Research Center of Engineering Technology For Utilization of Functional Ingredients From Botanicals (Hunan, China).

### 2.2. Sample collection and chemical analysis

On farrowing day, colostrum samples were drawn manually from every active teat of a sow after injection of 15 IU oxytocin. Blood samples (10 mL) were taken from ear veins into vacuum blood collection tubes on farrowing and d 40 of gestation. The blood samples were allowed to clot at room temperature for 30 min, and then centrifuged at  $3,000 \times g$  for 15 min at room temperature with the resulting serum stored at  $-20^\circ\text{C}$  until analysis (Liu et al., 2012). The activities of SOD, GSH-Px, catalase, nitric oxide synthetase (NOS) and MDA were determined by assay kits

**Table 1**  
Ingredient and chemical composition of basal diet (air-dry basis).

Ingredient, %	Content	Chemical composition, % of DM	Content
Corn	70.00	Digestible energy, MJ/kg DM	13.40
Soybean meal	11.20	Crude protein	13.10
Wheat bran	14.40	Lysine	0.60
Fish meal	1.60	Methionine	0.20
CaHPO <sub>4</sub>	1.10	Calcium	0.75
CaCO <sub>3</sub>	0.16	Total phosphorus	0.60
NaCl	0.40	Available-P	0.37
Premix <sup>1</sup>	1.14	Crude fiber	3.30
Total	100		

DM = dry matter.

<sup>1</sup> The premix provided the following per kilogram of diet: Cu5 mg, Fe 80 mg, Zn 50 mg, Mn 20 mg, Se 0.15 mg, I 0.14 mg, VA 4,000 IU, VD<sub>3</sub> 200 IU, VE 44 IU, VK<sub>3</sub> 4.00 mg, VB<sub>1</sub> 1.0 mg, VB<sub>2</sub> 3.75 mg, VB<sub>3</sub> 40.60 mg, pantothenic acid 20.0 mg, VB<sub>6</sub> 10 mg, VB<sub>12</sub> 0.015 μg, folic acid 12 mg, d-biotin 0.34 mg.

according to the manufacturer's instructions (Nanjing Jiancheng Technology LTD., China). The contents of serum hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitric oxide (NO) were also determined by assay kits in accordance with the protocols provided by the manufacture (Nanjing Jiancheng Technology LTD., China).

### 2.3. Statistical analysis

All data were analyzed by ANOVA using the GLM procedures of SPSS11.0. The statistical model consisted of the effect of diet. Pen was used as the experimental unit for the piglet performance data, whereas individual sow was used as the experimental unit for reproductive performance and blood analysis. Multiple comparisons were carried out by the Tukey test. Differences were considered significant when  $P < 0.05$ . The results are reported as the means and standard errors (means  $\pm$  SE).

## 3. Results

### 3.1. Litter performance

Sows received 200 and 300 mg catechin per kg diets had improved ( $P < 0.01$ ) pigs born alive and pig born health compared with sows fed the control diet. Sows received 200, 300, and 400 mg catechin per kg diets had reduced ( $P < 0.05$ ) stillborn compared with sows fed the control diet (Table 2).

### 3.2. Antioxidative status indicators in sow serum

The serum GSH-Px activity did not differ among all treatments on d 40 of gestation and at farrowing ( $P > 0.05$ ). But all levels of catechin improved ( $P < 0.05$ ) activities of sow serum SOD and CAT at farrowing. In addition, no significant changes ( $P > 0.05$ ) in sow serum NOS activity was observed on d 40 of gestation and at farrowing. At farrowing, serum MDA level decreased with the increase

of catechins and the lowest MDA content was observed in group III (200 mg/kg catechins) ( $P < 0.01$ ). And the serum MDA level in groups IV and V didn't show any significant difference compared with the group I even the content of diet catechins increased from 300 to 400 mg/kg. However, no effects ( $P > 0.05$ ) of catechins on serum MDA concentration were noticed on d 40 of gestation. Serum H<sub>2</sub>O<sub>2</sub> content decreased ( $P < 0.05$ ) with the increase of catechins on d 40 of gestation and at farrowing compared with the control. Similar to NOS activity in serum, there was no difference ( $P > 0.05$ ) in serum level of NO among treatments (Table 3).

## 4. Discussions

Oxidative stress leads to low reproductive performance through the pregnancy complications, preterm labor, fetal growth restriction, miscarriage or abnormal fetal development (Dennerly, 2004; Agarwal et al., 2003), which adversely affects the production of highly prolific sows (Berchieri-Ronchi et al., 2011). Previous results showed that oxidative stress led to both lipid and protein oxidation, impaired normal endothelial cell functions (Serdar et al., 2003), and altered placenta and fetal development as well (Hansen et al., 2001; Prater et al., 2008). Litter size at birth is determined by the implantation of fertilized eggs and fetal development in uterus during the early gestation, which directly affects the reproductive performance of breeding sows and production of their offsprings (Herrera and Ortega-Senovilla, 2010; Berchieri-Ronchi et al., 2011). Therefore, it is very important to decrease oxidative damage caused by excessive free radicals including reactive oxygen species (ROS) and nitrogen species (RONS) on fetal implantation and development in wombs of sows during the early gestation. One of the primary aims of this study was to investigate whether oxidative stress and its damage on the litter performance could be reduced by providing the catechins during the early gestation of sows. Our results demonstrated that catechins showed a beneficial effect on the sow reproductive performance, including significant improvements in

**Table 2**  
Effects of dietary catechins on litter performance.

Item	Group I	Group II	Group III	Group IV	Group V	P-value
Piglet born alive	8.70 $\pm$ 0.70 <sup>baB</sup>	7.60 $\pm$ 0.92 <sup>bb</sup>	11.60 $\pm$ 0.90 <sup>aA</sup>	11.40 $\pm$ 0.68 <sup>aA</sup>	10.10 $\pm$ 1.06 <sup>abAB</sup>	0.005
Piglet born healthy	7.70 $\pm$ 0.61 <sup>a</sup>	7.10 $\pm$ 0.79 <sup>a</sup>	10.70 $\pm$ 0.76 <sup>b</sup>	10.70 $\pm$ 0.62 <sup>b</sup>	8.70 $\pm$ 1.00 <sup>ab</sup>	0.001
Low birth weight piglets	0.58 $\pm$ 0.19	0.40 $\pm$ 0.22	0.91 $\pm$ 0.21	0.64 $\pm$ 0.31	1.33 $\pm$ 0.58	0.307
Stillborn	0.75 $\pm$ 0.25 <sup>a</sup>	0.30 $\pm$ 0.15 <sup>ab</sup>	0.09 $\pm$ 0.30 <sup>b</sup>	0.09 $\pm$ 0.30 <sup>b</sup>	0.22 $\pm$ 0.15 <sup>b</sup>	0.027
Birth weight, g	1578 $\pm$ 65.23	1533 $\pm$ 51.41	1524 $\pm$ 32.90	1653 $\pm$ 75.95	1564 $\pm$ 75.45	0.603

Values are means  $\pm$  SE,  $n = 12$ . Means within a row without common lower-case letters ( $P < 0.05$ ) and upper-case letters ( $P < 0.01$ ) differ.

**Table 3**  
Effects of catechins on antioxidant enzymes and radicals in serum of the pregnant sows.

Item	Period	Group I	Group II	Group III	Group IV	Group V	P-value
GSH-Px, U/mL	d 40 of gestation	277.8 $\pm$ 2.33	285.4 $\pm$ 8.56	293.1 $\pm$ 7.17	290.2 $\pm$ 6.21	258.3 $\pm$ 5.25	0.229
	Farrowing	300.0 $\pm$ 6.35	327.2 $\pm$ 12.61	319.0 $\pm$ 19.44	314.4 $\pm$ 11.33	290.1 $\pm$ 16.38	0.364
SOD, U/mL	d 40 of gestation	74.44 $\pm$ 1.37	75.12 $\pm$ 3.92	78.63 $\pm$ 3.28	78.43 $\pm$ 2.26	73.79 $\pm$ 6.04	0.824
	Farrowing	80.25 $\pm$ 3.00 <sup>b</sup>	90.38 $\pm$ 2.32 <sup>a</sup>	90.00 $\pm$ 2.82 <sup>a</sup>	91.79 $\pm$ 2.31 <sup>a</sup>	89.03 $\pm$ 2.69 <sup>a</sup>	0.021
CAT, U/mL	d 40 of gestation	2.74 $\pm$ 0.32	3.52 $\pm$ 0.57	3.66 $\pm$ 0.43	3.81 $\pm$ 0.20	4.06 $\pm$ 0.59	0.326
	Farrowing	2.91 $\pm$ 0.36 <sup>A</sup>	3.96 $\pm$ 0.54 <sup>B</sup>	4.34 $\pm$ 0.25 <sup>B</sup>	4.29 $\pm$ 0.23 <sup>B</sup>	5.14 $\pm$ 0.29 <sup>B</sup>	0.005
NOS, U/mL	d 40 of gestation	12.92 $\pm$ 0.71	12.83 $\pm$ 0.54	13.44 $\pm$ 0.54	13.72 $\pm$ 0.79	13.56 $\pm$ 0.65	0.830
	Farrowing	9.11 $\pm$ 0.58	9.32 $\pm$ 0.77	8.79 $\pm$ 0.17	9.21 $\pm$ 0.52	9.47 $\pm$ 0.62	0.932
MDA, nmol/mL	d 40 of gestation	7.21 $\pm$ 0.19	6.97 $\pm$ 0.14	6.84 $\pm$ 0.48	6.78 $\pm$ 0.33	7.08 $\pm$ 0.12	0.825
	Farrowing	7.67 $\pm$ 0.23 <sup>A</sup>	6.97 $\pm$ 0.26 <sup>A</sup>	5.92 $\pm$ 0.42 <sup>B</sup>	7.01 $\pm$ 0.27 <sup>A</sup>	6.94 $\pm$ 0.20 <sup>A</sup>	0.008
H <sub>2</sub> O <sub>2</sub> , nmol/L	d 40 of gestation	13.11 $\pm$ 0.51 <sup>A</sup>	11.75 $\pm$ 0.23 <sup>B</sup>	11.43 $\pm$ 0.22 <sup>B</sup>	11.00 $\pm$ 0.35 <sup>B</sup>	10.56 $\pm$ 0.22 <sup>B</sup>	0.000
	Farrowing	15.53 $\pm$ 1.62 <sup>a</sup>	12.69 $\pm$ 0.64 <sup>ab</sup>	11.36 $\pm$ 0.60 <sup>b</sup>	11.27 $\pm$ 0.51 <sup>b</sup>	11.03 $\pm$ 0.47 <sup>b</sup>	0.020
NO, $\mu$ mol/L	d 40 of gestation	3.50 $\pm$ 0.036	3.64 $\pm$ 0.31	4.12 $\pm$ 0.25	4.27 $\pm$ 0.31	4.33 $\pm$ 0.42	0.321
	Farrowing	2.87 $\pm$ 0.55	3.00 $\pm$ 0.20	3.18 $\pm$ 0.32	2.77 $\pm$ 0.07	2.90 $\pm$ 0.10	0.592

Values are means  $\pm$  SE,  $n = 12$ . Means within a row without common lower-case letters ( $P < 0.05$ ) and upper-case letters ( $P < 0.01$ ) differ.

GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; NOS = nitric oxide synthetase; MDA = malondialdehyde; H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; NO = nitric oxide.

the litter size (total and alive), piglet born healthy and average piglet birth weight, and a substantial decrease in stillborn. Additionally, sows receiving 200 or 300 mg catechins per kg diets showed higher litter performance compared with sows from other treatments, indicating that there may be a dose–effect relationship in a certain range between catechins and sow performances. Moreover, free radical-induced birth defects and other situations such as abortions, stillborn and mummies (Lagod et al., 2001; Loeken, 2004) could be avoided or decreased when the catechins is added into the gestation diets of sows during the early gestation.

Animals' antioxidant defense systems can cope with ROS including hydroxyl radicals, superoxide radicals, hydrogen peroxide and the oxidative metabolites such as MDA (Gielgiel et al., 2012). However, during the whole gestation, there is an increased energy demand and oxygen requirement with heavy metabolic burden, which favors a state of oxidative stress resulted from the overproduction of reactive oxygen species (Agarwal et al., 2003; Reyes et al., 2006). The excessive ROS and NOS and disrupted antioxidant system caused by the deficiency of antioxidant such as SOD, GSH-Px, CAT, NOS were reported to be involved in a variety of reproductive problems (Gupta et al., 2003; Sugino et al., 2007; Berchieri-Ronchi et al., 2011; Hana et al., 2015). This indicates that it may be necessary to provide or supplement some antioxidants such as vitamin E and vitamin A, fish oil, linseed oil and catechins from plant extracts in the diet during the gestational period in order to compensate for the substantial loss of these nutrients.

The effect of catechins on the above mentioned oxidative enzymes and oxidative metabolites including MDA, H<sub>2</sub>O<sub>2</sub> and NO were assessed in this trial. The results showed that, activities of SOD and CAT in serum of sows at farrowing were increased by 12 to 77% relative to the control, despite that only a numerical increase in activities of serum GSH-Px and NOS caused by dietary supplementation of catechins was observed in the present study. This result demonstrated that the anti-oxidant ability of sows at farrowing were mainly modulated by SOD and CAT, which consist of sows' defense system with other anti-oxidant enzymes in order to cope with the oxidative damage caused by excessive radicals. Corresponding to the change of serum enzyme activities of sows was the variation of oxidative metabolites such as MDA, H<sub>2</sub>O<sub>2</sub> and NO in serum of sows. Compared with the improvement on the serum SOD and CAT activities by catechins, both serum MDA and H<sub>2</sub>O<sub>2</sub> levels were significantly decreased on d 40 of gestation and at farrowing. This indicated that catechins had a good effect on the anti-oxidant ability of sows by increasing the serum enzyme activities and decreasing the levels of oxidative metabolites including MDA, H<sub>2</sub>O<sub>2</sub> and NO in serum of sows. The similar tendency of serum NO level and NOS activity as affected by catechins suggested a fact that the catechins had little effects on anti-oxidative capacity modulated through NOS and NO of sows under our experimental conditions.

## 5. Conclusions

Catechin had a positive effect on the reproductive performance, antioxidant and health status of sows when added into the diet during the early gestation. The optimal supplementation level is between 200 and 300 mg catechin per kg diet based on the relationship between catechin and reproductive performance and antioxidative indicators of gestation sows.

## Conflicts of interest

None of the authors have any conflict of interest to declare.

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