



## Original Research Article

## Tea polyphenols increase the antioxidant status of laying hens fed diets with different levels of ageing corn

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## ARTICLE INFO

## Article history:

Received 16 July 2020  
 Received in revised form  
 10 August 2020  
 Accepted 18 August 2020  
 Available online 29 April 2021

## Keywords:

Tea polyphenol  
 Antioxidant capacity  
 Laying hen  
 Ageing corn

## ABSTRACT

This study was conducted to evaluate the effects of ageing corn levels (stored for 4 years) with or without the supplementation of tea polyphenols (TPP) on the performance, egg quality and antioxidant status of laying hens. A total of 288 Lohmann commercial laying hens (63-week-old) were used under a 2 × 4 factorial arrangement with 4 levels of dietary ageing corn (0%, 25%, 50%, or 100%) and 2 levels of TPP (0 and 600 mg/kg) for 8 wk. Dietary ageing corn linearly decreased ( $P < 0.05$ ) the egg production, serum total antioxidant capacity (T-AOC), liver glutathione peroxidase (GSH-Px) of laying hens, yolk index, yolk colour, 1,1-diphenyl-2-picrylhydrazyl (DPPH) value and the reducing power value of egg yolk, but it linearly increased ( $P < 0.05$ ) the feed conversion rate, ovary malondialdehyde (MDA) content of laying hens, and the protein carbonyl content of egg yolk. Tea polyphenol supplementation increased ( $P < 0.05$ ) the serum T-AOC, serum superoxide dismutase (SOD), liver SOD, liver GSH-Px, ovary SOD, GSH-Px, the expression of antioxidant-related genes of laying hens, albumen height, Haugh unit, DPPH value and the majority free amino acids of egg yolk, but it decreased ( $P < 0.05$ ) the serum MDA content of laying hens, MDA and protein carbonyl of egg yolk. In conclusion, the ageing corn significantly reduced the performance, egg quality, antioxidant status and egg antioxidant capacity of laying hens, while TPP supplementation partially counteracted the adverse effects, especially antioxidant status and egg antioxidant capacity of laying hens.

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## 1. Introduction

Table eggs are readily available and an inexpensive source of nutrients that contain important macro-nutrients and micro-nutrients such as highly digestible proteins and lipids, mineral and vitamin (Attia et al., 2014; Nimalaratne et al., 2016). The

potential antioxidant capacity against oxidative stress from egg-derived compounds has attracted great attention (Davalos et al., 2004; Katayama et al., 2007). The extent of the antioxidant capacity is attributed to the antioxidant compounds including amino acids, egg protein, derived antioxidative peptides, xanthophylls, phospholipids, vitamin E and selenium of eggs (Botsoglou et al., 2012; Nimalaratne et al., 2016; Nimalaratne and Wu, 2015; Youssef et al., 2015).

Corn is the most crucial feedstuff in laying hen diet, which can be stored for a long time under suitable storage conditions. However, corn undergoes pronounced biochemical and nutritional changes during storage and then becomes the ageing corn with a high acidity value (Rehman et al., 2002; Zhou et al., 2002; Zia-Ur-Rehman, 2006). Lipid and protein peroxidation products in ageing corn can induce oxidative stress of animals, which are considered the main factors affect the health of animals (Wu et al., 2014; Yue et al., 2011). It has been reported that ageing corn reduced the

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



antioxidant status of broilers (Yin et al., 2017). In our previous study, 100% ageing corn in a diet reduced the laying rate, feed intake, egg weight, antioxidant status of laying hens, albumen height, yolk colour and polyunsaturated fatty acid of eggs. Vitamin E supplementation were unable to counteract the adverse effects (Mu et al., 2018; Zhou et al., 2019). Whether reducing the levels of ageing corn can alleviate the decreasing of antioxidant capacity of eggs and antioxidant status of laying hens are not clear.

Tea polyphenols (TPP) are a natural antioxidant, which could prevent the accumulation of reactive oxygen species and increase the antioxidant capacity of animals (Frei and Higdon, 2003). Studies on mice showed that TPP can increase the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and prevent peripheral insulin resistance, which is induced by free fatty acids (Yan et al., 2011). A previous study has reported that diets with 0.5% extract of green tea supplementation could significantly increase the feed intake, egg production and yolk index of laying hens (Ariana et al., 2011). It has been reported that the supplementation of TPP at 600 and 1,000 mg/kg can counteract the adverse effects of vanadium on the antioxidant capacity of laying hens, egg quality and antioxidant capacity of eggs (Yuan et al., 2016). Whether TPP supplementation could alleviate the decreasing antioxidant capacity of eggs and antioxidant status of laying hens fed ageing corn are not clear.

What levels of dietary ageing corn will cause the adverse effect and whether TPP supplementation can counteract the adverse effects of laying hens fed ageing corn are not clear. Therefore, we hypothesized that ageing corn would linearly decrease the performance, egg quality, antioxidant status and egg antioxidant capacity of laying hens, and TPP supplementation could partially counteract the adverse effects of ageing corn.

## 2. Materials and methods

### 2.1. Ethics statement

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

### 2.2. Tea polyphenols and ageing corn preparation

Tea polyphenols were purchased from Clooney Tea Technology (Co., Ltd. Ya'an, China) with 98.99% purity. It consists of 50.34% catechin, 45.62% epigallocatechin gallate, 1.45% caffeine and 3.37% moisture.

The ageing corn had been stored for 4 years in the national barns (Jilin, China). The regular corn had been stored for 6 months in a grain depot (Ningxia Province, China). For the ageing corn and regular corn samples, crude protein (CP), dry matter (DM), crude fat (CF) were determined as described by the AOAC International (2006). Filter bags and the fiber analyzer equipment (Fiber Analyzer, Ankom Technology, Macedon, NY, USA) were used to measure the neutral detergent fiber and acid detergent fiber according to Van Soest et al., 1991 with a slight modification. Acidity value was measured by following the national standard method (China GB/T 20570-2015; MAPRC, 2015). The activity of catalase and peroxidase were determined using specific reagent kits (Jiancheng Bioengineering Institute, Nanjing, PR China). The mycotoxins and xanthophylls were determined using a high-performance liquid chromatography (HPLC) instrument equipped with a fluorescence detector (Agilent 1100, Agilent Technologies, Santa Clara, CA, USA). Determination of the fatty acid profiles was carried out in regular and ageing corns by HPLC (China GB 5009.168-2016; MAPRC, 2016).

### 2.3. Birds and diets

The current study was performed in summer on a research farm in Ya'an, China. The study used a 4 × 2 factorial experimental design with 4 levels of ageing corn to replace the regular corn at 0%, 25%, 50% and 100% and with or without the supplementation of TPP at 600 mg/kg. Two hundred and eighty-eight 63-week-age Lohmann commercial laying hens were assigned to 1 of the 8 dietary treatments, with 6 replicates of 6 hens in 2 cages each. The temperature was maintained at 28 ± 4 °C and relative humidity at 50% to 65%. The photoperiod was set at 16L:8D. Diets were formulated to meet Agricultural Trade Standardization of China (NY/T33-2004; MAPRC, 2004) requirements for the requirements of all nutrients (Table 1). Hens were allowed ad libitum access to mash feed and water. The feeding trial lasted for 8 wk.

### 2.4. Hen performance

The egg production, egg weight, number of dirty and broken eggs were recorded daily. The laying rate, average egg weight, dirty egg rate and broken egg rate were calculated. Feed intake was recorded weekly and then feed conversion rate was calculated.

### 2.5. Egg collection

Till the termination of the trial, a total of 48 eggs per treatment with 8 eggs per replicate were collected and divided into 2 parts. A half of the eggs were used for the egg quality determination. The other half were used for the egg yolk antioxidant capacity determination.

### 2.6. Sample collection

On the last day of the trial, blood samples ( $n = 6$ ) were collected from the brachial vein of one laying hen per replicate and placed into lithium heparinized vacutainers. Serum was obtained at 4 °C and stored at -20 °C for antioxidant status analysis. Following blood collection, chicks were sacrificed by cervical dislocation, and liver and ovaries were quickly removed, frozen and powdered in -80 °C for Real-time PCR and antioxidant status analysis, respectively.

### 2.7. Egg quality

Multi-tester (EMT-7300, Robotmation Co., Ltd., Tokyo, Japan) was used to determine the albumen height, Haugh unit and the egg yolk colour. Egg yolk and eggshell (dried at room temperature for 3 d) were carefully separated and weighed. The proportions of eggshell, albumen and yolk in the egg were calculated. Vernier calipers were used to determine the eggshell thickness (without inner and outer shell membranes), which was defined as the average of 3 different points (top, middle, and bottom). Yolk index (the ratio height to width) was determined according to the method of (Funk, 1948). A texture analyzer (detector: 2P; crosshead speed: 2 mm/s; TA. XT. plus. Stable Microsystems) was used to determine the vitelline membrane strength.

### 2.8. Antioxidant status of laying hens

#### 2.8.1. Antioxidant enzyme activity assay

The liver and ovarian tissue samples ( $n = 6$ ) were homogenized in PBS and centrifuged at 1,699 × *g* for 10 min to obtain the supernatant. The activities of SOD and GSH-Px, the content

**Table 1**  
Ingredients and nutrient levels of the experimental diets (as fed basis).

Item	0% Ageing corn	25% Ageing corn	50% Ageing corn	100% Ageing corn
Ingredients, g/kg				
Regular corn	570.5	427.9	285.3	0.0
Ageing corn	0.0	142.6	285.3	570.5
Soybean meal	224.0	224.0	224.0	224.0
Wheat bran	78.1	78.1	78.1	78.1
Soybean oil	20.0	20.0	20.0	20.0
Calcium carbonate	8.3.5	8.3.5	8.3.5	8.3.5
Calcium hydrophosphate	11.2	11.2	11.2	11.2
L-Lysine-HCl	1.3	1.3	1.3	1.3
D,L-Methionine	1.1	1.1	1.1	1.1
Sodium chloride	4.0	4.0	4.0	4.0
Mineral premix <sup>1</sup>	5.0	5.0	5.0	5.0
Vitamin premix <sup>2</sup>	0.3	0.3	0.3	0.3
Choline chloride, 50%	1.0	1.0	1.0	1.0
Nutrient content <sup>3</sup> , g/kg				
Metabolizable energy, MJ/kg	11.09	11.09	11.09	11.09
Crude protein	155.0	155.0	155.0	155.0
Calcium	35.1	35.1	35.1	35.1
Available phosphorus	3.2	3.2	3.2	3.2
Lysine	7.9	7.9	7.9	7.9
Methionine	3.2	3.2	3.2	3.2
Threonine	5.2	5.2	5.2	5.2
Tryptophan	1.5	1.5	1.5	1.5

<sup>1</sup> Mineral premix provided the following per kilogram of the diet: iron, 60 mg; copper, 8 mg; manganese, 60 mg; zinc, 80 mg; selenium, 0.3 mg; iodine, 0.35 mg.

<sup>2</sup> Vitamin premix provided the following per kilogram of the diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 1,600 IU; vitamin E, 5 IU; vitamin B<sub>1</sub>, 0.8 mg; vitamin B<sub>2</sub>, 2.5 mg; vitamin B<sub>6</sub>, 1.5 mg; vitamin B<sub>12</sub>, 0.004 mg; D-pantothenic acid, 2.2 mg; folic acid, 0.25 mg; nicotinic acid, 20 mg; biotin, 0.1 mg.

<sup>3</sup> Calculated values.

malondialdehyde (MDA) in liver, ovarian tissue and serum were analyzed using reagent kits (Jiancheng Bioengineering Institute, Jiangsu, China).

### 2.8.2. Antioxidant-related genes expression

Total RNA was isolated from the liver and ovary using the Trizol reagent ( $n = 6$ ; Takara, Dalian, China) according to the manufacturer's protocol. The ratio of absorbance at 260 nm to that at 280 nm was used in each sample. The complementary DNA was synthesized using the primeScript RT reagent kit (Takara, Dalian, China) and real-time PCR was performed using the SYBR Premix Ex Tap (Takara, Dalian, China). PCR reactions took place on an Applied Biosystems 7900HT Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). All of these operations were according to the manufacturer's protocol and Zhang et al. (2020) Amplification cycles were performed with initial heating at 95 °C for 35 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 34 s, 95 °C for 15 s, and 60 for 15 s. Each mRNA level was expressed as its ratio to  $\beta$ -actin mRNA, and gene expression was calculated as  $2^{-\Delta\Delta Ct}$  method (Livak and Schmittgen, 2000). All real-time PCR were performed in triplicate in a 384-well plate. Sequences of primers antioxidant-related genes are presented in Table 2.

### 2.9. The antioxidant capacity of eggs

#### 2.9.1. Free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The scavenging effect of the DPPH free radical ( $n = 6$ ) was measured by the previous method (Zhang et al., 2011). The sample solution (1 mL, 10 mg egg yolk/mL; ethanol itself as control) was mixed with the same volume of DPPH (0.1 mmol/L) solution, shaken and left for 30 min at room temperature. The absorbance was determined at 517 nm. The scavenging effect was calculated according to the following equation:

**Table 2**

Gene-specific primers for real-time quantitative reverse transcription PCR.

Genes	Primers (5' to 3')
<i>Nrf2</i>	Forward: TGTGTGTGATTCAACCCGACT Reverse: TTAATGGAAGCCGCCACT
<i>NQO1</i>	Forward: GTTCAATGCCGTGCTCTCAC Reverse: CCGCTTCAATCTTCTTCTGC
<i>SOD3</i>	Forward: CCAGTGTGGCTGATAATGAGACT Reverse: CTATTTTGAGCTGGGCTTCA
<i>HO-1</i>	Forward: TTGGCAAGAAGCATCCAGA Reverse: TCCATCTCAAGGGCATTCA
<i>GCLC</i>	Forward: GGACAGGCACAGACACAGAA Reverse: TGCTGTGGATGAATCCCT
<i>GSTA3</i>	Forward: TTGGATAAGGCCGCAAACAGATA Reverse: TTCCAGTAAATGCACGTCTGCTA
<i>GSTT</i>	Forward: GACGGAGACTTACCCTAGCAGA Reverse: TGATGGGTACCAGTGGTACGGA

*Nrf2* = nuclear factor erythroid 2-related 2; *NQO1* = NAD(P)H: quinone dehydrogenase 1; *SOD3* = superoxide dismutases 3; *HO-1* = heme oxygenase-1; *GCLC* = glutathione cysteine ligase catalytic; *GSTA3* = glutathione S-transferase-A3; *GSTT* = glutathione S-transferase theta.

DPPH scavenging activity (%) = [(Blank absorbance – Sample absorbance)/Blank absorbance] × 100.

#### 2.9.2. Reducing power

Reducing power was determined according to the method of (Klompong et al., 2007) with slight modifications ( $n = 6$ ). A 0.5-mL sample solution (40 mg of egg yolk/mL) was added to 0.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. The mixture was mixed with the same volume of 10% trichloroacetic acid and centrifuged at 956 × *g* (Centrifuge 5430/R, Eppendorf, Hamburg, Germany)

at 4 °C for 10 min. The supernatant of solution (0.5 mL) was added to 0.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride, and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power.

### 2.9.3. Malondialdehyde content

The MDA content of egg yolk ( $n = 6$ ) was determined using specific reagent kits (Jiancheng Bioengineering Institute, Jiangsu, China). Briefly, yolk samples (0.5 g) were homogenized with 4.5 mL of absolute ethanol. Subsequently, 1 mL of the homogenate was treated with thiobarbituric acid, mixed rapidly and heated in a boiling water bath for 40 min. The samples were centrifuged at  $10,621 \times g$  for 10 min and the supernatants pipetted into a 96-well plate. The absorbance of the supernatant was measured at 532 nm.

### 2.9.4. Protein carbonyl content

The protein carbonyl content of egg yolk ( $n = 6$ ) was determined using specific reagent kits (Jiancheng Bioengineering Institute, Jiangsu, China). Briefly, the extracted samples were treated with a 2, 4-dinitrophenylhydrazine-HCl solution, then proteins were precipitated using trichloroacetic acid. The sediments were solubilized in a guanidine-HCl solution (6 mol/L) and the absorbance was measured at 370 nm. The results were defined as the nano-moles of carbonyl groups per milligram of soluble protein with a molar extinction coefficient of  $22,000 \text{ m}^2/\text{mol}$ . Soluble protein concentration was determined by the bicinchoninic acid method using a reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

## 2.10. The composition of egg yolk

### 2.10.1. Alpha-tocopherol

The  $\alpha$ -tocopherol content in egg yolks was measured by HPLC (Agilent Technologies 1260, Santa Clara, USA) according to (Botsoglou et al., 2012) with slight modifications ( $n = 6$ ). The chromatographic column (15 cm  $\times$  0.46 cm) contained Zorbax C18 (5  $\mu\text{m}$ ; Agilent, Santa Clara, CA, USA). The mobile phase was 100% methanol with a flow rate of 0.8 mL/min. The column effluents were set at 294 nm. The peak heights and a standard calibration curve were quantified as detector signals.

### 2.10.2. Free amino acid

Free amino acids were determined by the method according to (Nimalaratne et al., 2016) with slight modifications ( $n = 6$ ). Approximately 100 mg of freeze-dried egg yolk was extracted with 1 mL of 10% sulfosalicylic acid solution vortexing and centrifugation at  $15,294 \times g$  for 10 min (Centrifuge 5430/R, Eppendorf, Hamburg, Germany). The supernatant was filtered with a 0.22- $\mu\text{m}$  nylon syringe filter and analyzed by an automated AA analyzer (L-8900, Hitachi, Tokyo, Japan) with a lithium high-performance column.

### 2.10.3. Fatty acid profile

The analysis of the egg yolk ( $n = 6$ ) was measured according to the national standard method (China GB 5009.168-2016; MAPRC, 2016). The gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionisation detector and a silica capillary column (100 m by 0.25 mm i.d. by 0.20  $\mu\text{m}$  of film thickness; SP-2560, Supelco, Bellefonte, PA, USA) was used to separate the fatty acid methyl esters. The carrier gas was nitrogen with a pressure of 0.5 kg/cm<sup>2</sup>. The temperature of the injector and detector was 250 °C. The results were expressed in grams of fatty acids per 100 g of sample.

## 2.11. Statistical analyses

Data were analyzed by ANOVA in a 4  $\times$  2 factorial design using the GLM procedures of SPSS 23.0 (SPSS Inc., Chicago, IL). The main effects (ageing corn or TPP) and interactions between the 2 factors were carried out. Turkey's test was applied when any of the interactions showed significance. Each replicate was the experimental unit. Data were shown as the means and pooled SEM. The results were considered significantly different at  $P \leq 0.05$ . Linear contrast was included in the analysis to determine the animal response to increasing dietary ageing corn levels.

## 3. Results

### 3.1. Characterization of the experimental corn

The characteristics of ageing corn and regular corn are shown in Table 3. Ageing corn had the lowest moisture, crude protein, crude fiber, xanthophyll content and peroxidase and catalase activities compared with the regular corn. In addition, ageing corn had a higher acidity value, neutral detergent fiber, acid detergent fiber, ash and deoxynivalenol content compared with the regular corn.

### 3.2. Hen performance

Production performance data are shown in Tables 4 and 5. Dietary ageing corn did not affect the average daily feed intake (ADFI), however linearly ( $P = 0.024$ ) decreased the egg production from 5 to 8 weeks, egg weight from 1 to 4 weeks, feed conversion ratio (FCR) from 5 to 8 weeks and the whole period, and linearly trended to decrease the egg production ( $P = 0.097$ ) and egg weight ( $P = 0.092$ ) in the whole period. The supplementation of TPP did not affect production performance. No significant interaction between ageing corn  $\times$  TPP was observed.

**Table 3**  
Characteristics of the experimental corn used (g/kg, air-dried basis)<sup>1</sup>.

Item	Ageing corn	Regular corn
Moisture	130.6	143.1
Crude protein	77.3	75.6
Crude fat	33.1	34.2
Ash	25.6	9.9
Gross energy, cal/g	3,846.2	3,799.1
Xanthophylls, mg/kg	4.9	15.5
Neutral detergent fiber	123.5	107.3
Acid detergent fiber	100.8	98.1
Acidity of fatty acids, mg KOH/100 g	126.0	64.0
Malondialdehyde, nmol/mL	40.3	96.0
Protein carbonyl, nmol/mg protein	13.44	4.06
Catalase, U/mg	17.0	28.5
Peroxidase, U/mg	34.3	64.9
Aflatoxin B1, $\mu\text{g}/\text{kg}$	ND	ND
Zearalenone, $\mu\text{g}/\text{kg}$	ND	ND
Deoxynivalenol, $\mu\text{g}/\text{kg}$	240.90	ND
Fatty acid, mg/kg		
C16:0	2.5	3.2
C18:0	0.3	0.3
C18:1	3.9	5.7
C18:2	9.2	11.4
C18:3	<0.1	0.3

ND = not detected.

<sup>1</sup> Ageing corn: originating from the national barns in Jilin province, China, and stored for 4 years. Regular corn: originating from Ningxia province, China, and stored for 6 months.

**Table 4**  
Effect of tea polyphenols (TPP) on the production performance of laying hens fed diets with different levels of ageing corn<sup>1</sup>.

TPP, mg/kg	Ageing corn	Egg production, %			Egg weight, g			ADFI, g		
		1 to 4 wk	5 to 8 wk	1 to 8 wk	1 to 4 wk	5 to 8 wk	1 to 8 wk	1 to 4 wk	5 to 8 wk	1 to 8 wk
0	0%	84.33	85.22	84.77	64.64	64.24	64.44	120.59	112.43	116.51
0	25%	86.31	83.53	84.92	64.60	64.75	64.67	120.75	112.58	116.67
0	50%	86.21	84.33	85.27	64.22	65.05	64.62	121.18	116.78	118.98
0	100%	83.63	74.90	79.27	64.25	63.81	64.05	120.60	113.33	116.96
600	0%	88.00	86.91	87.45	64.91	64.13	64.52	119.04	113.21	116.13
600	25%	83.63	82.33	82.98	65.62	65.33	65.48	121.50	114.59	118.06
600	50%	88.79	80.56	84.67	64.29	64.41	64.34	120.14	113.53	116.84
600	100%	84.92	80.46	82.69	63.00	63.88	63.43	120.18	113.78	116.98
SEM		0.93	1.32	1.07	0.23	0.22	0.21	0.63	0.86	0.70
Main effect										
Ageing corn										
	0%	86.16	86.06 <sup>a</sup>	86.11	64.77 <sup>ab</sup>	64.19	64.48 <sup>ab</sup>	119.82	112.82	116.32
	25%	84.97	82.93 <sup>ab</sup>	83.95	65.11 <sup>a</sup>	65.04	65.08 <sup>a</sup>	121.13	113.59	117.36
	50%	87.50	82.44 <sup>ab</sup>	84.97	64.26 <sup>ab</sup>	64.73	64.48 <sup>ab</sup>	120.66	115.15	117.91
	100%	84.28	77.68 <sup>b</sup>	80.98	63.62 <sup>b</sup>	63.85	63.74 <sup>b</sup>	120.39	113.56	116.97
TPP, mg/kg										
	0	85.12	81.99	83.56	64.43	64.46	64.45	120.78	113.78	117.28
	600	86.33	82.56	84.45	64.45	64.44	64.44	120.22	113.78	117.00
P-value										
	Corn	0.631	0.178	0.375	0.124	0.246	0.193	0.903	0.804	0.875
	TPP	0.517	0.830	0.678	0.953	0.955	0.993	0.658	0.999	0.843
	Corn × TPP	0.646	0.634	0.781	0.371	0.811	0.676	0.927	0.727	0.845
	Linear effect of ageing corn	0.576	0.024	0.097	0.030	0.333	0.092	0.886	0.733	0.784

<sup>a, b</sup> Different superscripts in a row indicate a significance difference ( $P < 0.05$ ).

<sup>1</sup> Each value represents the mean value of 6 replicates per treatment.

**Table 5**  
Effect of tea polyphenols (TPP) on production performance of laying hens fed diets with different levels of ageing corn<sup>1</sup>.

TPP, mg/kg	Ageing corn	FCR			Dirty eggs, %			Broken eggs, %		
		1 to 4 wk	5 to 8 wk	1 to 8 wk	1 to 4 wk	5 to 8 wk	1 to 8 wk	1 to 4 wk	5 to 8 wk	1 to 8 wk
0	0%	2.22	2.06	2.14	2.05	2.09	2.07	0.47	0.83	0.65
0	25%	2.18	2.10	2.14	3.27	4.13	3.69	0.81	1.21	1.00
0	50%	2.20	2.15	2.17	4.02	5.40	4.69	1.07	1.09	1.08
0	100%	2.25	2.38	2.31	3.21	7.26	5.15	2.03	1.07	1.58
600	0%	2.09	2.04	2.06	2.93	4.13	3.53	1.37	1.65	1.51
600	25%	2.23	2.14	2.19	4.84	5.21	5.02	0.87	0.91	0.89
600	50%	2.12	2.27	2.18	2.67	5.73	4.13	0.55	0.69	0.59
600	100%	2.26	2.26	2.26	1.26	3.48	2.34	0.94	1.12	1.03
SEM		0.03	0.04	0.03	0.31	0.48	0.35	0.17	0.15	0.14
Main effect										
Ageing corn										
	0%	2.16	2.05	2.10	2.49	3.11	2.80	0.92	1.24	1.08
	25%	2.21	2.12	2.16	4.06	4.67	4.36	0.84	1.06	0.95
	50%	2.16	2.21	2.18	3.34	5.57	4.41	0.81	0.89	0.84
	100%	2.26	2.32	2.28	2.23	5.37	3.75	1.48	1.10	1.30
TPP, mg/kg										
	0	2.22	2.17	2.19	3.14	4.72	3.90	1.10	1.05	1.08
	600	2.17	2.18	2.17	2.92	4.64	3.75	0.93	1.09	1.00
P-value										
	Corn	0.552	0.157	0.281	0.160	0.274	0.352	0.491	0.874	0.647
	TPP	0.462	0.938	0.762	0.731	0.931	0.839	0.639	0.889	0.783
	Corn × TPP	0.628	0.794	0.910	0.145	0.167	0.129	0.232	0.463	0.245
	Linear effect of ageing corn	0.247	0.017	0.043	0.047	0.121	0.530	0.214	0.784	0.496

<sup>1</sup> Each value represents the mean value of 6 replicates per treatment.

### 3.3. Egg quality

The egg quality is shown in Table 6. Dietary ageing corn linearly ( $P = 0.005$ ) decreased yolk index and yolk colour, and trended ( $P = 0.056$ ) to linearly decrease the albumen height. The supplementation of TPP significantly ( $P = 0.026$ ) increased the albumen height and Haugh unit. No significant interaction between ageing corn × TPP was observed.

### 3.4. Antioxidant status of laying hens

#### 3.4.1. Antioxidant enzyme activity assay

The antioxidant enzyme activity in the serum, liver and ovary are shown in Table 7. Dietary ageing corn linearly decreased the serum T-AOC ( $P = 0.043$ ) and liver GSH-Px ( $P = 0.002$ ), and increased ( $P = 0.018$ ) the ovary MDA content. The supplementation of TPP increased the serum T-AOC ( $P = 0.044$ ) and SOD ( $P = 0.002$ ),

**Table 6**  
Effect of tea polyphenols (TPP) on egg quality of laying hens fed diets with different levels of ageing corn (8 wk)<sup>1</sup>.

TPP, mg/kg	Ageing corn	Egg shell strength, N	Eggshell thickness, mm	Albumen height, mm	Haugh unit	Yolk index	Yolk colour	Vitelline membrane strength, g of force
0	0%	0.035	0.35	9.46	95.30	0.41	7.82	5.03
0	25%	0.031	0.34	9.49	95.66	0.40	7.23	5.16
0	50%	0.032	0.35	9.57	95.78	0.40	7.27	5.24
0	100%	0.034	0.36	8.94	93.18	0.38	5.19	5.19
600	0%	0.034	0.36	9.99	97.90	0.40	7.69	5.52
600	25%	0.034	0.35	9.66	96.34	0.39	6.90	5.48
600	50%	0.030	0.35	9.57	95.78	0.40	6.50	4.92
600	100%	0.031	0.34	9.65	97.02	0.39	5.33	5.25
SEM		<0.01	<0.01	0.08	0.37	<0.01	0.08	0.10
Main effect								
Ageing corn								
	0%	0.034	0.36	9.72	96.60	0.40 <sup>a</sup>	7.76 <sup>a</sup>	5.27
	25%	0.032	0.34	9.57	96.00	0.39 <sup>ab</sup>	7.06 <sup>ab</sup>	5.32
	50%	0.031	0.35	9.57	95.78	0.40 <sup>a</sup>	6.89 <sup>ab</sup>	5.08
	100%	0.032	0.35	9.29	95.10	0.38 <sup>b</sup>	5.26 <sup>b</sup>	5.22
TPP, mg/kg								
	0	0.033	0.35	9.36 <sup>b</sup>	94.98 <sup>b</sup>	0.40	0.11	5.15
	600	0.032	0.35	9.72 <sup>a</sup>	96.76 <sup>a</sup>	0.39	0.11	5.29
P-value								
	Corn	0.447	0.323	0.279	0.551	0.006	<0.001	0.829
	TPP	0.605	0.460	0.026	0.017	0.634	0.072	0.470
	Corn × TPP	0.329	0.098	0.369	0.239	0.386	0.177	0.462
	Linear effect of ageing corn	0.380	0.549	0.056	0.158	0.005	<0.001	0.724

<sup>a, b</sup> Different superscripts in a row indicate a significance difference ( $P < 0.05$ ).  
<sup>1</sup> Each value represents the mean value of 6 replicates per treatment (4 eggs per replicate).

**Table 7**  
Effect of tea polyphenols (TPP) on antioxidant status of laying hens fed diets with different levels of ageing corn<sup>1</sup>.

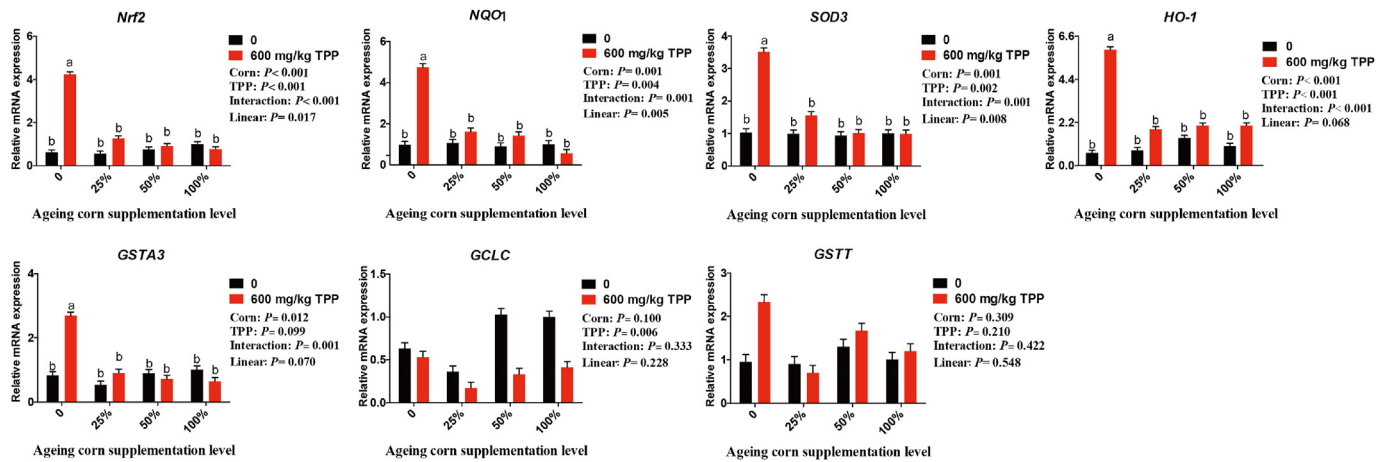
TPP, mg/kg	Ageing corn	Serum				Liver				Ovary			
		T-AOC, U/mg prot	SOD, U/mg prot	MDA, nmol/mg prot	GSH-Px, U/mg prot	T-AOC, U/mg prot	SOD, U/mg prot	MDA, nmol/mg prot	GSH-Px, U/mg prot	T-AOC, U/mg prot	SOD, U/mg prot	MDA, nmol/mg prot	GSH-Px, U/mg prot
0	0%	1.33	311.10	15.17	ND	1.15	117.82	0.85	28.48 <sup>b</sup>	0.71	56.34	1.14	71.68
0	25%	1.13	317.86	19.38	ND	1.22	120.68	0.81	28.30 <sup>b</sup>	0.56	55.74	1.13	64.65
0	50%	0.87	293.14	15.32	ND	1.26	116.54	1.00	27.00 <sup>b</sup>	0.67	74.29	1.66	69.75
0	100%	1.07	296.74	20.52	ND	1.22	110.86	0.91	27.22 <sup>b</sup>	0.56	53.98	1.58	54.92
600	0%	1.57	342.78	8.87	ND	1.24	132.08	0.61	46.03 <sup>a</sup>	0.77	87.29	1.06	89.46
600	25%	1.47	346.93	9.25	ND	1.33	143.05	0.62	50.42 <sup>a</sup>	0.58	85.39	1.06	90.50
600	50%	1.40	352.88	10.77	ND	1.18	122.11	0.93	30.25 <sup>b</sup>	0.52	89.74	1.51	86.38
600	100%	0.91	311.29	10.46	ND	1.29	124.16	0.91	26.17 <sup>b</sup>	0.52	65.63	1.54	79.98
SEM		0.06	5.12	1.01		0.05	3.40	0.04	1.26	0.05	4.81	0.07	2.95
Main effect													
Ageing corn													
	0%	1.45 <sup>a</sup>	326.94	12.02		1.20	124.95	0.73 <sup>b</sup>	37.25 <sup>a</sup>	0.74	71.82	1.10 <sup>b</sup>	80.57
	25%	1.30 <sup>ab</sup>	332.40	14.32		1.28	131.87	0.71 <sup>b</sup>	39.36 <sup>a</sup>	0.57	70.56	1.10 <sup>b</sup>	77.58
	50%	1.14 <sup>ab</sup>	323.01	13.04		1.22	119.32	0.97 <sup>a</sup>	28.63 <sup>b</sup>	0.59	82.02	1.59 <sup>a</sup>	78.06
	100%	0.99 <sup>b</sup>	304.02	15.49		1.25	117.51	0.91 <sup>ab</sup>	26.70 <sup>b</sup>	0.54	59.80	1.56 <sup>a</sup>	67.45
TPP, mg/kg													
	0	1.10 <sup>b</sup>	304.71 <sup>b</sup>	17.60 <sup>a</sup>		1.21	116.48 <sup>b</sup>	0.89	27.75 <sup>b</sup>	0.62	60.09 <sup>b</sup>	1.38	65.25 <sup>b</sup>
	600	1.34 <sup>a</sup>	338.47 <sup>a</sup>	9.84 <sup>b</sup>		1.26	130.35 <sup>a</sup>	0.77	38.22 <sup>a</sup>	0.60	82.01 <sup>a</sup>	1.29	86.58 <sup>a</sup>
P-value													
	Corn	0.043	0.243	0.642		0.931	0.449	0.027	0.002	0.468	0.453	0.018	0.420
	TPP	0.044	0.002	<0.001		0.618	0.048	0.084	<0.001	0.787	0.028	0.552	0.001
	Corn × TPP	0.216	0.475	0.698		0.898	0.857	0.609	0.006	0.879	0.853	0.994	0.919
	Linear effect of Ageing corn	0.007	0.092	0.342		0.797	0.254	0.342	0.006	0.186	0.422	0.007	0.147

T-AOC = total antioxidant capacity; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; prot = protein; ND = not detected.  
<sup>a, b</sup> Different superscripts in a row indicate a significance difference ( $P < 0.05$ ).  
<sup>1</sup> Each value represents the mean value of 6 replicates per treatment.

liver SOD ( $P = 0.048$ ), ovary SOD ( $P = 0.028$ ) and GSH-Px ( $P < 0.001$ ), but decreased the serum MDA content ( $P < 0.001$ ). An interaction was found between ageing corn and TPP on the liver GSH-Px ( $P = 0.006$ ) in which 100% and 50% ageing corn groups with the supplementation of TPP significantly decreased the activity of GSH-Px compared with 0% and 25% ageing corn groups and TPP supplementation could counteract the adverse effect.

### 3.4.2. Antioxidant-related genes expression

The gene expression in liver and ovary are shown in [Figs. 1 and 2](#). The ageing corn × TPP interactions were observed for liver superoxide dismutase (*SOD3*), NAD(P)H quinone dehydrogenase 1 (*NQO1*,  $P = 0.005$ ), nuclear factor erythroid 2-related 2 (*Nrf2*,  $P = 0.017$ ), heme oxygenase-1 (*HO-1*,  $P < 0.001$ ) and glutathione S-transferase-A3 (*GSTA3*,  $P = 0.001$ ) expression in



**Fig. 1.** Effect of tea polyphenols (TPP) on gene expression in the liver of laying hens fed diets with different levels of ageing corn. Data were acquired using real-time PCR. Values are means and SEM. Each value represents the mean value of 6 replicates per treatment. <sup>a, b</sup> Different superscripts in bars indicate a significance difference ( $P < 0.05$ ). *Nrf2* = nuclear factor erythroid 2-related 2; *NQO1* = NAD(P)H: quinone dehydrogenase 1; *SOD3* = superoxide dismutases 3; *HO-1* = heme oxygenase-1; *GSTA3* = glutathione S-transferase-A3; *GCLC* = glutathione cysteine ligase catalytic; *GSTT* = glutathione S-transferase theta.

which layers fed regular corn with the supplementation of TPP had the highest liver *SOD3*, *NQO1*, *Nrf2*, and *GSTA3* compared with the other groups. The supplementation of TPP significantly increased the liver glutathione cysteine ligase catalytic (*GCLC*) expression ( $P = 0.006$ ). Both ageing corn and TPP did not affect the ovary *GCLC*, *SOD3*, *NQO1*, *Nrf2*, glutathione S-transferase theta (*GSTT*) and *HO-1* expression ( $P > 0.05$ ). The supplementation of TPP significantly increased the ovary *GSTA3* expression ( $P = 0.022$ ).

### 3.5. Antioxidant capacity of eggs

The antioxidant capacity of eggs is shown in Table 8. Dietary ageing corn linearly ( $P = 0.002$ ) decreased the values of DPPH and reducing power, but linearly ( $P < 0.001$ ) increased the protein carbonyl content of egg yolks. Moreover, the DPPH value was increased ( $P = 0.001$ ), but the MDA ( $P = 0.022$ ) and protein carbonyl ( $P = 0.011$ ) were decreased by the TPP supplementation.

### 3.6. The composition of egg yolk

#### 3.6.1. Alpha-tocopherol

There was no difference in  $\alpha$ -tocopherol among the diet treatments (Table 8).

#### 3.6.2. Free amino acids

The free amino acids are shown in Table 9. Ageing corn linearly ( $P = 0.016$ ) decreased the content of threonine. The group fed no ageing corn had the highest ( $P = 0.007$ ) threonine content compared with 50% and 100% ageing corn groups. Groups of 0% and 25% ageing corn had a higher serine content. The group of 25% ageing corn had the highest glycine ( $P = 0.018$ ), alanine ( $P = 0.007$ ) and phenylalanine ( $P = 0.015$ ) content. The group of 100% ageing corn had the lowest lysine ( $P = 0.020$ ) content. The group of 50% ageing corn had the lowest ( $P = 0.005$ ) histidine content. TPP supplementation increased ( $P < 0.001$ ) the content of threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, lysine and histidine in egg yolks.

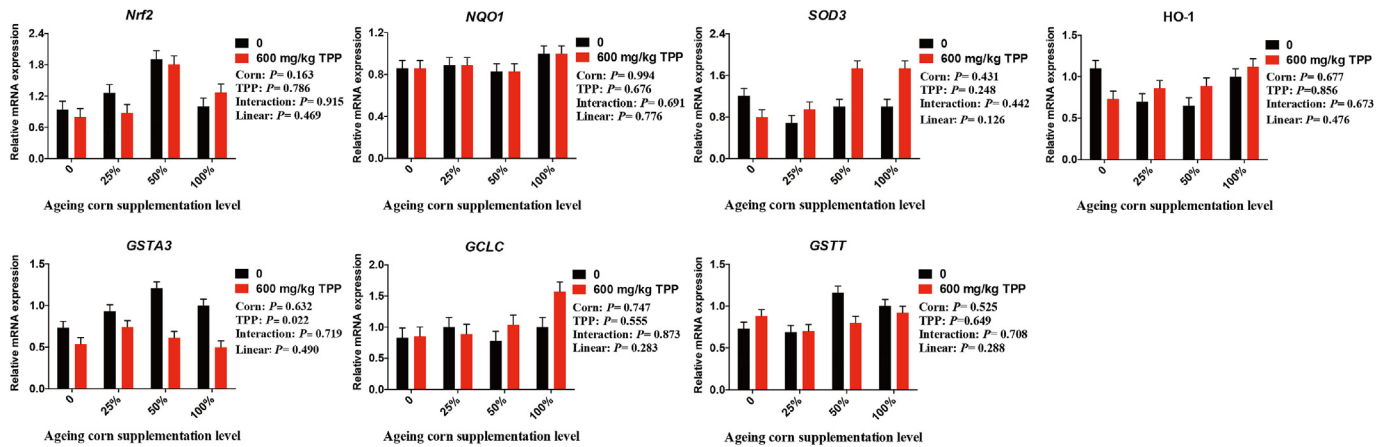
#### 3.6.3. Fatty acid profile

In general, ageing corn tended to linearly decrease the n-6 ( $P = 0.081$ ), n-3 ( $P = 0.087$ ) and polyunsaturated fatty acid ( $P = 0.077$ ) contents in egg yolks (Table 10).

## 4. Discussion

During corn ageing, a series of physicochemical and physiological changes occur (Zhou et al., 2002). Acidity value is the most important parameter to evaluate the corn quality during storage. The increased acidity of ageing corn could be ascribed to the increasing concentration of free fatty acids and phosphate, which led to corn deterioration (Morrison, 2006). Previous studies have shown that the oxidation of lipids and proteins results in the producing of hydroperoxides and advanced oxidation protein products in ageing corn (Baskol et al., 2008; Wang et al., 2012), and the activities of peroxidase and catalase were decreased during grains storage (Yin et al., 2017; Zhou et al., 2002). These parameters can be used to evaluate corn quality during storage. Meanwhile, the ageing corn was originated from the national barns, where the storage condition was suitable and mycotoxin contamination was not found in ageing. The changes of nutritional components, such as lipids and protein peroxidation products in ageing corn, were the main factors to cause adverse effects.

In the current study, ageing corn linearly depressed the egg production and increased the FCR from 5 to 8 wk. Although the egg production was not different among the 50%, 25% and 0% ageing corn groups, the egg production of 50% and 25% ageing corn groups was still decreased by 3.61% and 3.13% from 5 to 8 wk, and 1.14% and 2.13% in the whole period compared with the regular corn group, respectively. The higher acidity value indicated that lipid and protein peroxidation were occurred during corn storage, and lipid and protein peroxidation products were produced during corn storage. The consumption of ageing corn increased the expose of laying hen to cumulative oxidative byproducts, resulting in an impaired redox balance and repressed production performance (Wu et al., 2014; Yue et al., 2011). The results indicated that the adverse effects by the cumulative oxidative byproducts increased with the increase of feeding time and quantity of ageing corn. The level of ageing corn to replace the regular corn at less than 25% should be further investigated in laying hen diets. Our result was in agreement with the previous study by Mu et al. (2018) who reported that dietary ageing



**Fig. 2.** Effect of tea polyphenols (TPP) on gene expression in the ovary of laying hens fed diets with different levels of ageing corn. Data were acquired using real-time PCR. Values are means and SEM. Each value represents the mean value of 6 replicates per treatment. *Nrf2* = nuclear factor erythroid 2-related 2; *NQO1* = NAD(P)H: quinone dehydrogenase 1; *SOD3* = superoxide dismutases 3; *HO-1* = heme oxygenase-1; *GSTA3* = glutathione S-transferase-A3; *GCLC* = glutathione cysteine ligase catalytic; *GSTT* = glutathione S-transferase theta.

corn repressed the egg production and FCR of laying hens. Moreover, Yuan et al. (2016) observed that feeding 600 mg/kg TPP can increase egg production in vanadium-containing diets of laying hens. Our result was not in accordance with it, which indicated that TPP was unable to counteract the decreased egg production of layers fed ageing corn.

The protein quality of corn was adversely affected as a result of storage (Angel et al., 2003; Zia-Ur-Rehman, 2006). The higher protein carbonyl content in ageing corn indicated that protein quality was decreased during corn storage. The reduction of Haugh unit in ageing corn groups may resulted from the worse protein quality of ageing corn for blocking the generation of albumen compared with the regular corn. Yolk index could evaluate the egg freshness. Our results showed that dietary ageing corn linearly decreased the yolk index. A previous study reported that vitelline

membrane strength was significantly related to the yolk index (Kirunda and Mckee, 2000). However, there was no difference in vitelline membrane strength. This needs to be further investigated. In the current study, TPP supplementation could increase the albumen height and Haugh unit, which was in accordance with our previous research (Yuan et al., 2016). As hypothesized, TPP could increase the egg quality by the prevention of oxidation but was unable to do so in this research and to counteract the its adverse effects when laying hens were fed ageing corn.

The typical yellow colour of the yolk is given by the accumulation of lutein and zeaxanthin from the diets (Oliveira and Rodriguez-Amaya, 2007). Birds cannot synthesize lutein and zeaxanthin, and therefore, they must obtained from the diet (Blount et al., 2000). Corn is rich in lutein and zeaxanthin, which is prone to degradation during storage (Moros et al., 2002). Our

**Table 8**  
Effect of tea polyphenols (TPP) on egg yolk antioxidant capacity of laying hens fed diets with different levels of ageing corn<sup>1</sup>.

TPP, mg/kg	Ageing corn	DPPH, %	Reducing power (A <sub>700 nm</sub> ) <sup>2</sup>	Malondialdehyde, nmol/g	Protein carbonyl, nmol/mg protein	α-tocopherol, mg/100 g
0	0%	24.67	0.30 <sup>a</sup>	129.256	37.49	1.51
0	25%	20.61	0.25 <sup>cd</sup>	175.974	40.62	1.69
0	50%	15.97	0.26 <sup>bcd</sup>	137.071	42.51	1.65
0	100%	16.67	0.24 <sup>d</sup>	157.87	44.94	1.60
600	0%	29.93	0.28 <sup>ab</sup>	96.919	31.46	1.95
600	25%	24.38	0.27 <sup>bc</sup>	135.463	33.54	1.69
600	50%	25.60	0.24 <sup>d</sup>	138.683	44.13	1.55
600	100%	20.93	0.25 <sup>cd</sup>	103.965	42.11	1.91
SEM		0.78	<0.01	5.89	0.75	0.06
<b>Main effect</b>						
<b>Ageing corn</b>						
	0%	27.30 <sup>a</sup>	0.29 <sup>a</sup>	113.09 <sup>b</sup>	34.47 <sup>b</sup>	1.73
	25%	22.50 <sup>a</sup>	0.26 <sup>a</sup>	155.72 <sup>a</sup>	37.08 <sup>b</sup>	1.69
	50%	20.78 <sup>a</sup>	0.25 <sup>a</sup>	137.88 <sup>ab</sup>	43.32 <sup>a</sup>	1.60
	100%	18.80 <sup>b</sup>	0.25 <sup>b</sup>	130.92 <sup>ab</sup>	43.52 <sup>a</sup>	1.76
<b>TPP, mg/kg</b>						
	0	19.48 <sup>b</sup>	0.26	150.043 <sup>a</sup>	41.39 <sup>a</sup>	1.61
	600	25.21 <sup>a</sup>	0.26	118.758 <sup>b</sup>	37.81 <sup>b</sup>	1.78
<b>P-value</b>						
	Corn	0.003	<0.001	0.104	<0.001	0.775
	TPP	0.001	0.726	0.011	0.022	0.156
	Corn × TPP	0.543	0.043	0.388	0.185	0.297
	Linear effect of ageing corn	0.002	<0.001	0.721	<0.001	0.876

<sup>a, b</sup> Different superscripts in a row indicate a significance difference ( $P < 0.05$ ).

<sup>1</sup> Each value represents the mean value of 6 replicates per treatment (4 pooled egg yolks per replicate).

<sup>2</sup> The absorbance obtained at a wavelength of 700 nm.



**Table 9** Effect of tea polyphenols (TPP) on egg yolk free amino acids of laying hens fed diets with different levels of ageing corn (µg/g dry egg yolk)<sup>1</sup>.

TPP, mg/kg	Ageing corn	Ile	Leu	Tyr	Phe	Trp	Lys	His	Arg	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met
0	0%	286.35	528.65	400.18	400.85	114.06	573.12	87.85	523.94	272.83	244.72	428.50	1,067.66 <sup>b</sup>	147.30	230.06	374.01	190.45
0	25%	316.82	610.37	456.32	440.77	128.18	653.23	92.72	598.70	252.84	247.40	458.02	837.75 <sup>c</sup>	175.99	283.73	409.22	190.55
0	50%	257.19	513.44	393.56	372.75	109.24	526.72	73.93	478.62	224.51	208.96	386.83	752.52 <sup>c</sup>	145.77	228.45	336.62	162.04
0	100%	280.06	547.41	442.36	382.54	123.14	538.00	77.62	494.88	224.79	174.89	318.63	638.58 <sup>c</sup>	150.97	230.03	337.70	165.71
600	0%	369.40	666.74	486.71	499.66	123.96	675.87	82.48	573.50	259.85	321.87	547.28	1,226.44 <sup>ab</sup>	164.66	244.40	437.16	261.62
600	25%	394.00	680.77	499.43	560.83	134.15	740.29	101.95	544.01	197.58	284.44	635.33	1,415.43 <sup>a</sup>	176.97	263.81	513.98	318.52
600	50%	369.69	626.15	503.85	501.23	146.21	717.88	58.64	456.09	226.07	259.49	561.30	1,308.96 <sup>a</sup>	152.98	220.19	474.77	299.14
600	100%	378.83	666.70	550.59	501.20	127.56	635.29	88.58	630.48	252.48	286.91	585.80	1,390.00 <sup>a</sup>	175.64	266.91	511.49	302.43
SEM		6.48	10.65	9.19	7.44	3.50	12.03	2.74	22.18	10.87	5.89	11.38	24.95	3.00	4.89	8.61	6.09
Main effect																	
Ageing corn																	
0%		327.87	597.70	443.45	450.25 <sup>b</sup>	119.01	624.49 <sup>b</sup>	85.16 <sup>b</sup>	548.72	266.34	283.29 <sup>a</sup>	487.89 <sup>a</sup>	1,147.05	155.98 <sup>b</sup>	237.23 <sup>b</sup>	405.58	226.03
25%		355.41	645.57	477.87	500.80 <sup>b</sup>	131.16	696.76 <sup>b</sup>	97.33 <sup>b</sup>	571.35	225.21	265.92 <sup>ab</sup>	546.67 <sup>a</sup>	1,236.59	176.48 <sup>a</sup>	273.77 <sup>a</sup>	461.60	294.54
50%		313.44	569.80	448.70	436.99 <sup>b</sup>	127.73	622.30 <sup>b</sup>	66.28 <sup>a</sup>	467.36	225.29	234.23 <sup>b</sup>	474.06 <sup>b</sup>	1,030.74	149.37 <sup>b</sup>	224.32 <sup>b</sup>	405.70	230.59
100%		329.45	607.06	496.48	441.87 <sup>b</sup>	125.35	586.64 <sup>a</sup>	83.10 <sup>b</sup>	562.68	238.64	230.90 <sup>b</sup>	452.21 <sup>b</sup>	1,024.29	163.31 <sup>ab</sup>	248.47 <sup>ab</sup>	424.60	234.07
TPP, mg/kg		285.11 <sup>b</sup>	549.97 <sup>b</sup>	423.10 <sup>b</sup>	399.23 <sup>b</sup>	118.66 <sup>b</sup>	572.77 <sup>b</sup>	83.03	524.03	243.74	218.99 <sup>b</sup>	398.00 <sup>b</sup>	829.12 <sup>b</sup>	155.01	243.06	364.39 <sup>b</sup>	177.19 <sup>b</sup>
0		377.98 <sup>a</sup>	660.09 <sup>a</sup>	510.15 <sup>a</sup>	515.73 <sup>a</sup>	132.97 <sup>a</sup>	692.33 <sup>a</sup>	82.91	551.02	234.00	288.18 <sup>a</sup>	582.43 <sup>a</sup>	1,335.21 <sup>a</sup>	167.56	248.83	484.35 <sup>a</sup>	295.43 <sup>a</sup>
P-value																	
Corn		0.159	0.107	0.154	0.015	0.652	0.020	0.003	0.338	0.494	0.007	0.035	0.198	0.018	0.007	0.087	0.374
TPP		<0.001	<0.001	<0.001	<0.001	0.048	<0.001	0.983	0.546	0.656	<0.001	<0.001	<0.001	0.043	0.559	<0.001	<0.001
Corn × TPP		0.769	0.719	0.546	0.908	0.321	0.402	0.282	0.446	0.600	0.134	0.160	0.002	0.517	0.193	0.149	0.185
Linear effect of ageing corn																	
		0.712	0.787	0.156	0.309	0.737	0.130	0.284	0.965	0.523	0.016	0.240	0.300	0.978	0.871	0.992	0.973

<sup>a, b</sup> Different superscripts in a row indicate a significance difference ( $P < 0.05$ ).

<sup>1</sup> Each value represents the mean value of 6 replicates per treatment (4 pooled egg yolks per replicate).

**Table 10**

Effect of tea polyphenols (TPP) on fatty acid profile (g/100 g egg yolk) of laying hens fed diets with different levels of ageing corn<sup>1</sup>.

TPP, mg/kg	Ageing corn	SFA	MUFA	n-6 PUFA	n-3 PUFA	PUFA	USFA
0	0%	9.34	10.77	5.54	0.55	6.14	16.96
0	25%	9.08	10.41	5.68	0.55	6.29	16.75
0	50%	8.86	10.19	5.31	0.49	5.85	16.09
0	100%	9.23	10.58	5.22	0.51	5.78	16.40
600	0%	9.02	10.39	5.65	0.54	6.25	16.69
600	25%	8.95	10.36	5.53	0.51	6.10	16.50
600	50%	9.50	10.89	5.67	0.54	6.26	17.20
600	100%	9.27	10.81	5.09	0.50	5.64	16.50
SEM		0.10	0.14	0.11	0.01	0.12	0.19
Main effect							
Ageing corn							
	0%	9.18	10.58	5.60	0.54	6.19	16.83
	25%	9.02	10.38	5.61	0.53	6.19	16.63
	50%	9.18	10.54	5.49	0.51	6.06	16.64
	100%	9.25	10.69	5.15	0.51	5.71	16.45
TPP, mg/kg							
	0	9.13	10.49	5.44	0.53	6.02	16.55
	600	9.18	10.61	5.49	0.52	6.06	16.72
P-value							
	Corn	0.870	0.887	0.430	0.354	0.426	0.912
	TPP	0.793	0.665	0.828	0.771	0.841	0.643
	Corn × TPP	0.405	0.583	0.820	0.221	0.783	0.533
	Linear effect of ageing corn	0.627	0.618	0.081	0.087	0.077	0.465

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. USFA = unsaturated fatty acids.

<sup>1</sup> Each value represents the mean value of 6 replicates per treatment (4 pooled egg yolks per replicate).

results indicated the ageing corn had a lower xanthophylls content (4.9 vs. 15.5 mg/kg). The decreased yolk colour indicated that lutein and zeaxanthin degradation occurred in ageing corn during storage, and then decreased the deposition of lutein and zeaxanthin in eggs. Though the differences were not significant, 50% and 25% ageing corn groups decreased the yolk colour slightly compared with the regular corn group, and TPP could not alleviate the adverse effect.

During corn storage, the oxidation of lipids produced hydroperoxides and the oxidation of proteins produced advanced oxidation protein products causing the feed to contain various levels of oxidants (Baskol et al., 2008; Wang et al., 2012; Warnants et al., 1996). When the ingestion of the excess oxidants overwhelms the cellular antioxidant defense system, oxidative stress occurs (Ray et al., 2012). GSH-Px is an important antioxidant enzyme that can convert hydrogen peroxide into water. MDA is the most lipid peroxidation marker (Attia et al., 2020; Schieber and Chandel, 2014). In the current research, we found that serum T-AOC and liver GSH-Px were decreased, and MDA contents in liver and ovary were increased in the ageing corn groups. Our result was consistent with the previous study that feeding ageing corn to laying hens resulted in oxidative stress (Mu et al., 2018; Zhou et al., 2019).

TPP is a natural antioxidant. Our result suggests that TPP could increase the activities of antioxidant enzymes and decrease the MDA content of laying hens. Coimbra et al. (2006) reported that drink 1 L of green tea daily for 4 weeks could reduce the serum levels of MDA (by 30%), MDA+4-hydroxy-2(E)-nonenal (by 39%), the value of membrane-bound hemoglobin (by 25%) and improved band 3 profile (an erythrocyte transmembrane protein) of humans. Yuan et al. (2016) observed that the supplementation of TPP significantly decreased the activity of glutathione S-transferase of laying hens. TPP ameliorating oxidative status is considered via mediating Kelch-like ECH-associated protein-1/Nrf2 transcriptional pathways and regulating the expression of downstream

enzymes (Qi et al., 2017), and our results support this view. The interaction between ageing corn and TPP on liver GSH-Px indicated that TPP could partially alleviate the oxidative stress triggered by ageing corn.

*Nrf2* is the most important transcription factor in resistance to oxidative stress, which could activate a set of drug-metabolizing enzymes, such as glutathione S-transferases (*GST*) and *NQO1* by antioxidants and electrophiles (Szklarz, 2013). In our study, the expression of liver *Nrf2*, *GST* and *NQO1* showed a significant reduction in regular corn with the supplementation of TPP group. Patel and Maru (2008) observed that the drug-metabolizing enzymes such as *NQO1* and *GST* can be activated via the *Nrf2* pathway in the liver and lung of mice, and our results support this view. Superoxide is produced by losing one electron of molecular oxygen, and the accumulation of superoxide can damage and inactivate proteins containing iron-sulfur clusters. SOD can prevent the accumulation by converting superoxide into  $H_2O_2$  in the cell (Schieber and Chandel, 2014). Our results showed that TPP can increase the expression of *SOD3*, and then increase the antioxidant capacity of laying hens. Interesting, there were no difference between treatments in ovary gene expression, except *GSTA3*. This indicated that the antioxidant capacity of TPP will be reduced in the ovary.

DPPH and reducing power values were widely used to evaluate the antioxidant capacity of food. The linear reduction of DPPH and reducing power values indicated that dietary ageing corn could decrease the antioxidant capacity of eggs. Meanwhile, the TPP supplementation could counteract the adverse effects by increasing DPPH and reducing power values. We also noted that ageing corn groups linearly increased the protein carbonyl content in the yolk. This could be attributed to the higher protein carbonyl from ageing corn that transferred into yolk. Furthermore, the content of MDA and protein carbonyl were decreased by the TPP supplementation. Our results are in accordance with the finding reports by Wang et al. (2020) who reported that TPP increased the DPPH value and decreased MDA and protein carbonyl contents of eggs. Antioxidative compounds such as egg-derived peptides, tryptophan, tyrosine, vitamin E, carotenoids contribute to the total antioxidant capacity of egg yolk (Nimalaratne and Wu, 2015). A previous study reported that frying, boiling and microwaving would decrease the antioxidant capacity due to the degradation of antioxidative compounds during cooking (Nimalaratne et al., 2011). Similar, storage would lead to the degradation of protein and vitamin in the corn. Layers fed the ageing corn may have a lower content of vitamin, amino acids or other antioxidative compounds, resulting in lower antioxidant capacity of eggs. Thus, we next evaluate the content of antioxidative compounds in the eggs. In the current study, we found that dietary ageing corn linearly decreased the lysine in egg yolk, which may be ascribed to the degradation of amino acids in ageing corn. Moreover, we found that most amino acids were increased by the TPP supplementation, including 2 high antioxidant activity amino acids, tryptophan and tyrosine (Nimalaratne et al., 2011). This may explain the higher antioxidant capacity of eggs in the TPP groups. However, why TPP can improve the content of free amino acids is not clear and the reason may be that TPP prevents amino acids from oxidation (Chaudhury et al., 2015; Yang et al., 2018). The previous studies have demonstrated that fatty acid compositions of egg yolk closely reflected the variation in dietary fatty acid composition (Botsoglou et al., 2012; Cherian et al., 2007; Gładkowski et al., 2011). Polyunsaturated fatty acids are prone to oxidation during storage (Xin et al., 2019). The lower polyunsaturated fatty acids in ageing corn reflect the lower polyunsaturated fatty acids in egg yolks.

## 5. Conclusion

The present study demonstrated that the use of ageing corn (0%, 25%, 50%, 100%) with a high acidity (126 mg KOH/100 g) but low mycotoxin contamination linearly decreased the egg production, egg quality and antioxidant status of laying hens and antioxidant capacity of eggs. TPP (600 mg/kg) supplementation partially alleviates the adverse effects, which were reflected by increasing the activity of antioxidant enzymes, upregulating the expression of antioxidant-related genes of laying hens and increasing the free amino acids of egg yolks.

## Author contributions

**Ling Zhou:** conceptualization, data curation, methodology, writing – original draft preparation. **Xuemei Ding and Jianping Wang:** conceptualization. **Yue Xuan:** methodology. **Zuwei Su:** methodology. **Qiufeng Zeng and Shiping Bai:** formal analysis. **Keying Zhang:** conceptualization, writing – review & editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

## Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

## Acknowledgement

This work was financially supported by the National Science and Technology Project (2014BAD13B04), Sichuan Provincial Science and Technology Project (2018NZ20009).

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