Dietary naringin supplementation on laying performance and antioxidant capacity of Three-Yellow breeder hens during the late laying period

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ABSTRACT In this study, the effects of 3 graded dietary levels (0.1%, 0.2%, and 0.4%) of naringin were studied in Three-Yellow breeder hens during the late laying period (55−62 wk). A total of 480 Three-Yellow breeder hens (54-wk-old) were randomly divided into 4 groups (6 replicates of 20 hens): basal diet group (C), and basal diets supplemented with 0.1%, 0.2%, and 0.4% of naringin (N1, N2, and N3), respectively. Results showed that dietary supplementation with 0.1%, 0.2%, and 0.4% of naringin for 8 wk increased the laying rate and egg mass, enhanced egg yolk color, and decreased the feed egg ratio ($P < 0.05$). Meanwhile, compared with hens in C group, there were more preovulatory follicles and higher ovarian index as well as an enhanced ovarian somatic cell proliferation in hens of N2 and N3 groups ($P < 0.05$). With 0.2% and 0.4% naringin, glutathione concentration, the activity of catalase and total superoxide dismutase, and the total antioxidant capacity of ovarian tissues and serum increased ($P < 0.05$), while the contents of malondialdehyde and hydrogen peroxide decreased ($P < 0.05$). Moreover, compared to C group, the transcription levels of antioxidant genes in ovarian tissues increased in hens from N2 and N3 groups ($P < 0.05$). In conclusion, supplementation with 0.2% and 0.4% naringin both could improve the laying rate, ovarian and serum antioxidant capacity of Three-Yellow breeder hens during the late laying period.

Key words: naringin, Three-Yellow breeder hen, late laying period, laying performance, antioxidant capacity

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INTRODUCTION

Three-Yellow chicken is one of yellow broilers in China, which is famous for its delicious meat and high carcass quality. Normally, egg laying rate of Three-Yellow chicken reaches the peak (≥70%) at about 26 wk of age and lasts for few weeks, however, the laying rate is less than 55% at 50 wk of age. Moreover, the hen only produces about 77 eggs per year on average (Xu and Chen, 2003). Short laying period and low laying performance seriously reduce the number of breeding eggs and the commercial value of Three-Yellow chickens. Therefore, the exploration of strategies to prolong the laying period is necessary for improving the economic benefits of Three-Yellow chickens.

During the late laying period of hens, the egg laying rate declines sharply (Dai et al., 2020) due to age-related changes in hormone (Liu et al., 2013), ovarian dysfunction (May-Panloup et al., 2016), liver function failure (Huang et al., 2021a), etc. Numerous studies on laying hens demonstrated that ovarian function decline is one of the most crucial factors causing reductions in laying performance (Alvarez and Hocking 2012; Hao et al., 2020). It is demonstrated that at the late laying stage of the hens, decreased number of preovulatory follicles and growing follicles result in declines in laying performance and the level of serum estrogen (Zhang et al., 2021; Wu et al., 2022; Yao et al., 2022).

Growing evidences demonstrate that ovarian functions are associated with serum hormones, organism physiological state, ovarian redox state (Wu et al., 2017; Ren et al., 2018; Huang et al., 2021b), etc. In mammals, oxidative stress could increase the percentage of atretic follicles and decrease the secretion of reproductive hormones (Sharma et al., 2015; Yu et al., 2019). Similar to mammals, ovarian dysfunction induced by oxidative stress was found in laying hens and resulted in the decrease of laying performance and egg quality (Wang

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et al., 2016; Abbas et al., 2020; Wang et al., 2021a). Li et al. (2020) reported that oxidative stress caused by heat stress induces ovarian cells apoptosis, and leads to declines in the number of follicles and laying rate.

Recently, plant-derived extracts including polyphenols, carotenoids and flavonoids as antioxidants were added to the dietary in order to enhance the laying performance and egg quality by improving the resistance to oxidative stress of hens (Liu et al., 2014; Yuan et al., 2016; An et al., 2019). Naringin is one kind of flavonoids that is widely distributed in foods of plant sources such as vegetables and fruits, especially in the pomelo, grapefruit, and citrus rutaceae (Zeng et al., 2019). Studies on mice showed that naringin has effects of anti-inflammatory, antiapoptotic, neuroprotective, and antioxidant (Chtourou et al., 2016; Kim et al., 2018; Lim et al., 2018; Wang et al., 2021b). A previous study in broilers showed that naringin could improve liver antioxidant capacities to against thiram-induced tibial dyschondroplasia (Jiang et al., 2020). Goliomytis et al. (2015) demonstrated that naringin could improve the antioxidant capacity of breast and thigh meat in broilers. Besides, Hager-Theodorides et al. (2021) reported that naringin could up-regulated transcription levels of antioxidant genes, thereby improving antioxidant capacity of hens. However, the effects of naringin on laying performance of Three-Yellow breeder hens in the late laying stage remain poorly understood.

In the present study, it was designed to investigate the effects of dietary supplemented with naringin on laying performance, egg quality and antioxidant capacity on Three-Yellow breeder hens in the late laying stage. The results of this study would be helpful to prolong laying lifespan and provide theoretical basis for improving the egg production of Three-Yellow breeder hens.

MATERIALS AND METHODS

Birds and Experimental Design

All animal experiments in this study were performed in accordance with the recommendations of *Council for International Organizations of Medical Sciences* for animal use and were approved by the *Animal Care and Use Committee on the Ethics of Animal Experiments* of Guangxi University (NO. GXU2020-011).

A total of 480 healthy Three-Yellow breeder hens were raised in a local farm in Yulin (Guangxi Zhuang Autonomous Region, China). All hens were randomly allocated into control and naringin groups (0.1%, 0.2%, and 0.4%) with 6 replicates of 20 birds per replicate at 54 wk of age. Before that, a 2-wk pre-experiment was conducted to ensure that all hens used in this study were in the same status of health and laying rate. The hens in control group (**C**) were fed with basal diet (Table 1), which met all NRC requirements. The hens in 3 naringin groups (N1, N2, and N3) received a basal diet supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively. Naringin (purity ≥ 95%) used in this study was purchased from ChengDu ConBon Biotech Co. Ltd., (Chengdu, China).

Table 1. Ingredient and nutrient composition of the basal diet (air-dried basis, %).

Ingredients	Content (%)	Nutrient levels	Content (%)
Corn	63.50	ME/(MJ/kg)	11.49
Soybean meal	21.50	Crude protein	16.27
Soybean oil	2.50	Crude fat	4.66
Limestone	8.00	Crude fibre	1.62
Dicalcium phosphate	1.50	Calcium	3.39
NaCl	0.35	Total phosphorus	0.54
DL-Methionine	0.15	Methionine	0.40
Premix	2.5	Lysine	0.69
Total	100.00		

The premix provided the following (per kilogram of diet): vitamin A, 10,800 IU; vitamin D3, 2160 IU; vitamin E, 27 IU; vitamin K3, 1.4 mg; vitamin B1, 1.8 mg; vitamin B2, 8 mg; nicotinic acid, 32 mg; pantothenic acid, 11 mg; vitamin B6, 4.1 mg; folic acid, 1.08 mg; vitamin B12, 0.010 mg; biotin, 0.18 mg; Fe, 72 mg; Zn, 72 mg; Mn, 90 mg; Cu, 7mg; I, 0.9 mg; and Se, 0.27 mg.

All hens were housed in an individual wire cages (width: 30 cm, depth: 40 cm, height: 40 cm) with temperature between 23°C and 26°C, the relative humidity between 65 and 75%, and illumination at 16 h/D. Each of the 20 hens formed one experimental unit and shared a common feed trough. Hens were provided ad libitum access to water and feed. Eggs were collected at 10 am and 4 pm every day.

Sample Collection and Analytical Determination

Laying Performance The total number of eggs and the weight of each egg in each replicate were recorded every day. According to the production records, laying performance parameters include egg laying rate, egg mass, and average egg weight were calculated. The average daily feed intake (**ADFI**) was determined weekly by subtracting the final feed weight of each replicate from the initial feed weight. The feed egg ratio was calculated and recorded weekly (daily feed consumption/average egg weight).

Egg Quality At the midterm and the end of the experiment, 72 normal eggs were randomly selected (18 eggs from each group, 3 eggs per replicate) to evaluate egg quality traits. The eggshell thickness (mm) was measured using a micrometer (SanLiang Precision instrument Co. Ltd., Guangdong, China) at 3 different locations (sharp end, middle, and blunt end) of the egg. Eggshell strength (Pa), albumen height (mm), yolk color, egg yolk weight (g), and Haugh units were determined using an EA-01 egg analyzer (ORKA Food Technology, Ltd., Ramat Hasharon, Israel) according to the instructions of the manufacturer. The longitudinal and transverse diameters of the egg were measured via a vernier caliper (BiaoKang Science and Technology Co. Ltd., Shenzhen, China).

Sample Collection At the midterm and the end of the experiment, 3 hens in each replicate were randomly selected (18 hens per group) and weighed. Blood samples were collected from the wine vein, than centrifuged at 3,500 rpm for 10 min. The serum samples were stored at -80°C for antioxidant parameters analysis.

At the end of the experiment, 2 hens in each replicate (12 hens per group) were selected randomly. The hens were slaughtered by jugular exsanguination postanesthesia. Ovaries without preovulatory follicles (diameter >12 mm) were weighed. Ovaries without follicles over 1 mm in diameter immediately snap frozen in liquid nitrogen for the analysis of the antioxidant parameters, and the extraction of RNA and protein.

Morphological Observation of the Ovary

Ovaries without follicles over 1 mm in diameter were dissected and fixed in 4% neutral paraformaldehyde solution at 4°C for more than 48 h. After that, ovarian tissues were dehydrated in a graded ethanol and cleared in xylene. Subsequently, ovarian tissues were embedded in paraffin, sectioned into 5 μm thickness using a microtome (Leica Biosystems, Buffalo Grove, IL). The sections were deparaffinized in xylene and rehydrated in a graded dilutions of ethanol, and then stained with hematoxylin and eosin (**HE**).

Ovarian Somatic Cell Proliferation Analysis

Ovarian tissues were homogenized in ice-cold RIPA and then centrifuged at 12,000 rpm for 20 min at 4°C to obtain the supernatant. Protein concentration in supernatant was determined using a BCA protein assay kit (AR1189, Boster Biological Technology Co. Ltd., Wuhan, China). Around 20 μg protein from each sample were electrophoresed on a 10% SDS-PAGE, and then transferred to a polyvinylidene difluoride (**PVDF**) membrane. After blocked with 5% skimmed milk at room temperature for 2 h, the blots were probed with corresponding primary antibodies β-actin (1:1000; BM3873), PCNA (1:1000; BM0104) and CDK2 (1:1000; PB0562) purchased from Boster Biological Technology Co. Ltd., (Wuhan, China) with optimized conditions, and then incubated with the secondary antibody. Secondary horseradish peroxidase-conjugated goat anti-rabbit (1:5000; BA1054) and goat anti-mouse (1:5000; BA1050) antibodies were purchased from Boster Biological Technology Co. Ltd., (Wuhan, China). Immunological signals were detected by enhanced chemiluminescence (ECL) Kit using a ChemiScope 3400 Mini machine. The band intensities were quantified using Quantity one software and the results were normalized to β-actin.

Ovarian and Serum Antioxidant Capacity Determination

Ovarian tissues were homogenized in PBS, and centrifuged at 3,000 rpm for 10 min at 4°C to obtain 10% ovarian tissue homogenate. The protein concentration in homogenate was determined via a BCA protein assay kit (AR1189, Boster Biological Technology CO. Ltd., Wuhan, China). The activities of total superoxide dismutase (T-SOD; A015-1), catalase (CAT; A007-1), and total antioxidant capacity (T-AOC; A015-1), and the concentration of glutathione (GSH; A006-2), the malondialdehyde (MDA; A003-1) and hydrogen peroxide (H_2O_2; A064-1) in ovarian tissue homogenate and serum were determined using specific detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Quantitative Real-Time PCR (qRT-PCR) Analysis

Total RNA was isolated from ovarian tissues using RNAiso Plus (AJ11050A, TaKaRa Biotechnology, Dalian, China). RNA concentration was quantified depending on the absorbance at the wavelength of 260/280 nm using a NanoDrop 1000 UV spectrophotometer. 1.0 μg RNA was immediately used for reverse transcription using HiScript III RT SuperMix for qPCR (+gDNA wiper) reagent kits (R323-01,Vazyme Biotech Co., Ltd, Nanjing, China). qRT-PCR was performed using ChamQ Universal SYBR qPCR Master Mix (Q711-03, Vazyme Biotech Co. Ltd., Nanjing, China) according to the instructions of the manufacturer. Comparisons of transcription levels were determined by $2^{-\Delta\Delta Ct}$ formula method normalized to β-actin, and results were expressed as fold changes in gene transcription level over the control group. The sequences for forward and reverse primers are listed in Table 2.

Table 2. Primers used for qRT-PCR.

Gene name	Accession number	Primer sequence (5'-3')	Product length/bp
SOD1	NM_205064.2	F:GGCAATGTGACTGCAAAGGG R:CCCCTCTACCCAGGTCATCA	133
GPX1	NM_001277853.3	F:AGTACATCATCTGGTCGCCG R:CTCGATGTCGTCCTGCAGTT	137
CAT	NM_001031215.2	F:TCAGGAGATGTGCAGCGTTT R:TCTTACACAGCCTTTGGCGT	109
GLRX	NM_205160.2	F:GGATCCTGCCCTTACTGCAA R: GTTCGCTGCCCTGTTGTTTT	137
GSR	XM_040671422.1	F: TCCTGACTACGGCTTCGAGA R: AACTTGCCGTAACCACGGAT	150
PRDX3	XM_040674879.1	F:CAAGGGGAAATACCTCGTGCT R: CACCTCGCAGTTCACATCGT	118
β-actin	NM_205518.1	F:ACACCCACACCCCTGTGATGAA R:TGCTGCTGACACCTTCACCATTC	136

Abbreviations: *CAT*, catalase; *GLR*, glutaredoxin; *GPX1*, glutathione peroxidase 1; *GSR*, glutathione reductase; PRDX3, peroxiredoxin 3; *SOD1*, superoxide dismutase 1.

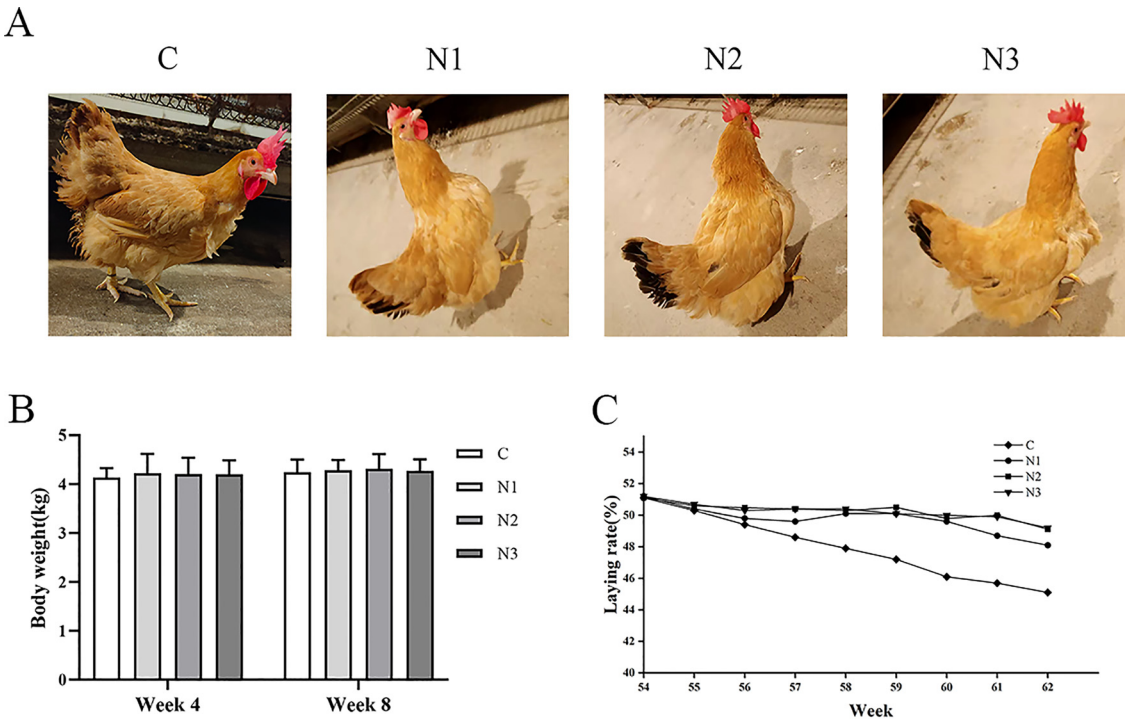
Statistical Analysis

All data were analyzed using SPSS 19.0 (SPSS. Inc., Chicago, IL). Differences in naringin diet supplementation were tested using one-way analysis of variance (**ANOVA**) for independent samples, followed by Duncan's multiple comparison tests. Polynomial contrasts for the linear and quadratic responses were used to determine the effect of different naringin levels. All data are presented as the means with standard error of mean (**SEM**). Values were considered to be statistically significant at $P < 0.05$.

RESULTS

Appearance, Body Weight, and Laying Rate

The results showed that dietary supplemented with naringin had no remarkable effect on the appearance of Three-Yellow breeder hens (Figure 1A). Meanwhile, dietary supplemented with naringin did not alter the body weight of Three-Yellow breeder hens ($P > 0.05$; Figure 1B). Dietary naringin supplementation had no effect on laying rate of hens from 55 to 57 wk of age ($P > 0.05$), but there was a linear increase in laying rate of



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	Egg-laying rate (%)							
Group	55W	56W	57W	58W	59W	60W	61W	62W
C	50.3	49.4	48.6	47.9[a]	47.2[a]	46.1[a]	45.7[a]	45.1[a]
N1	50.4	49.8	49.6	50.1[b]	50.1[b]	49.6[b]	48.7[b]	48.1[b]
N2	50.6	50.5	50.4	50.3[b]	50.5[b]	49.8[b]	50.0[c]	49.1[c]
N3	50.7	50.3	50.4	50.4[b]	50.1[b]	50.0[b]	49.9[c]	49.2[c]
SEM	1.170	1.185	0.801	0.776	0.736	0.692	0.551	0.400
P-value								
ANOVA	0.987	0.775	0.105	0.014	<0.001	<0.001	<0.001	<0.001
Linear	0.721	0.356	0.023	0.006	0.001	<0.001	<0.001	<0.001
Quadratic	0.990	0.718	0.358	0.073	0.004	0.002	<0.001	<0.001

[a, b]Mean values within a column with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 6). Abbreviations: C, basal diet group; N1, N2 and N3, basal diets supplemented with 0.1%, 0.2% and 0.4% of naringin, respectively; SEM, standard errors of mean.

Figure 1. Appearance, body weight, and laying rate of Three-Yellow breeder hens. [a,b]Mean values with different superscripts letters denote statistical significantly difference ($P < 0.05$). (A) Appearance of Three-Yellow breeder hens in C N1, N2, and N3. (B) Body weight (kg) of Three-Yellow breeder hens in each group after 4 and 8 wk of feeding (n = 12). (C,D) laying rate (%) of Three-Yellow breeder hens from 55 to 62 wk of age in each group (n = 6). Abbreviations: C, basal diet group; N1, N2, and N3, basal diet supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively.

hens at 58 wk ($P = 0.014$). Compared with C group, laying rate in the 3 treatment groups increased significantly ($P < 0.05$). During 59 to 60 wk, laying rate increased quadratically ($P < 0.05$). From 61 wk of age, laying rate was a significantly quadratic increase in hens from 3 treatment groups, compared with those in C group. Meanwhile, laying rate of hens in N2 and N3 groups were significantly higher than that in N1 group ($P < 0.05$; Figures 1C and 1D).

Laying Performance

As shown in Table 3, dietary supplemented with naringin did not have any effects on average egg weight and ADFI ($P > 0.05$). Compared with C group, at 55 to 61 wk, the egg mass of hens in 3 treatment groups were linearly increased ($P < 0.05$), meanwhile, the feed egg ratio in N1, N2, and N3 groups linearly decreased ($P < 0.05$). At 59 to 62 wk and 55 to 62 wk, dietary supplemented with naringin of 3 doses (0.1%, 0.2%, and 0.4%) enhanced quadratically in egg mass, and quadratically decreased feed egg ratio ($P < 0.05$). No significant difference was found in egg mass or feed egg ratio among 3 treatment groups ($P > 0.05$).

Egg Quality

As shown in Table 4, the results of egg quality at the midterm and the end of the experiment indicated that dietary supplemented with naringin had no significant effect on egg quality parameters include egg shape index, eggshell thickness, eggshell strength, albumen height, egg yolk weight, and Haugh unit ($P > 0.05$). After feeding with naringin for 8 wk, the egg yolk color was quadratically improved ($P < 0.05$). No significant difference was found in egg yolk color among 3 doses of naringin groups ($P > 0.05$, Table 4).

Table 3. Laying performance of Three-Yellow breeder hens.

	Groups					*P*-value		
Item	C	N1	N2	N3	SEM	ANOVA	Linear	Quadratic
55−58 W								
Laying rate (%)	49.05[a]	49.98[b]	50.45[b]	50.45[b]	0.393	0.012	0.003	0.124
Egg mass (g/d/hen)	22.97[a]	23.64[b]	23.89[b]	23.81[b]	0.245	0.011	0.004	0.051
Average egg weight (g)	46.84	46.98	46.95	46.95	0.099	0.513	0.326	0.336
ADFI (g/d)	80.75	81.00	80.73	80.65	0.174	0.259	0.317	0.211
Feed egg ratio	3.52[a]	3.45[b]	3.41[b]	3.40[b]	0.029	0.006	0.001	0.187
59−62 W								
Laying rate (%)	46.03[a]	49.13[b]	49.85[b]	49.80[b]	0.511	<0.001	<0.001	0.001
Egg mass (g/d/hen)	21.58[a]	23.60[b]	23.74[b]	23.57[b]	0.344	<0.001	<0.001	0.001
Average egg weight (g)	47.05	46.95	46.98	46.98	0.080	0.639	0.447	0.396
ADFI (g/d)	80.75	80.65	80.70	80.85	0.199	0.777	0.588	0.392
Feed egg ratio	3.74[a]	3.51[b]	3.42[b]	3.46[b]	0.051	<0.001	<0.001	0.003
55−62 W								
Laying rate (%)	47.54[a]	49.55[b]	50.15[b]	50.13[b]	0.528	<0.001	<0.001	0.011
Egg mass (g/d/hen)	22.27[a]	23.62[b]	23.82[b]	23.69[b]	0.273	<0.001	<0.001	0.01
Average egg weight (g)	46.94	46.96	46.97	46.96	0.066	0.987	0.775	0.831
ADFI (g/d)	80.75	80.83	80.71	80.64	0.142	0.618	0.325	0.462
Feed egg ratio	3.63[a]	3.48[b]	3.42[b]	3.43[b]	0.042	<0.001	<0.001	0.010

[a-b]Mean values within a row with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 6). Abbreviations: ADFI, Average daily feed intake ; C, basal diet group; N1, N2, and N3, basal diets supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively.

Table 4. Egg quality of Three-Yellow breeder hens.

	Groups					*P*-value		
Item	C	N1	N2	N3	SEM	ANOVA	Linear	Quadratic
58 W								
Egg shape index	1.29	1.28	1.28	1.28	0.015	0.895	0.914	0.891
Eggshell thickness (mm)	0.40	0.39	0.38	0.38	0.013	0.472	0.210	0.349
Eggshell strength (N)	42.58	42. 48	42.50	42.58	1.658	1.000	0.993	0.930
Albumen height (mm)	5.40	5.41	5.41	5.42	0.086	0.998	0.969	0.854
Egg yolk color	6.78	6.89	6.94	7.00	0.379	0.945	0.549	0.918
Egg yolk weight (g)	16.17	16.18	16.21	16.24	0.391	0.998	0.892	0.919
Haugh unit	77.16	77.23	77.21	77.19	0.659	1.000	0.977	0.927
62 W								
Egg shape index	1.27	1.27	1.28	1.26	0.015	0.667	0.556	0.483
Eggshell thickness (mm)	0.33	0.33	0.35	0.35	0.011	0.329	0.115	0.775
Eggshell strength (N)	41.48	41.56	41.46	41.58	2.810	1.000	0.982	0.992
Albumen height (mm)	5.39	5.43	5.38	5.37	0.231	0.993	0.861	0.889
Egg yolk color	7.28[a]	8.28[b]	8.50[b]	8.51[b]	0.286	<0.001	<0.001	0.016
Egg yolk weight (g)	16.08	16.05	16.10	16.06	0.318	0.999	0.971	0.979
Haugh unit	76.56	76.68	76.76	76.69	1.832	1.000	0.936	0.944

[a,b]Mean values within a row with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 18). Abbreviations: C, basal diet group; N1, N2, and N3, basal diets supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively.

Ovarian Morphology, Index, and Cell Proliferation

More preovulatory follicles were observed in hens supplemented with naringin compared to hens fed with basal diet (Figures 2A and 2B). The histomorphology of ovarian tissues showed that there were more growing follicles in ovaries from hens in 3 treatment groups, compared with those in C group (Figure 2C). The ovarian index of N2, and N3 groups were higher than that in the C group ($P < 0.05$). No significant difference was found in ovarian index between C and N1 group, or among 3 treatment groups (Figure 2D). Meanwhile, the expression levels of proteins related to cell proliferation PCNA and CDK2 in ovarian tissues of N2 and N3 groups were higher than those in C group ($P < 0.05$). A higher expression level of PCNA was observed in N1 group in comparison to C group ($P < 0.05$). However, no significant difference was detected in CDK2 expression between C and N1 group (Figures 2E and 2F).

Ovarian Antioxidant Capacity

Compared to the C group, the 3 treatment groups increased linearly in the activities of T-SOD and T-AOC of ovarian tissues, and decreased linearly in the concentration of H_2O_2 ($P < 0.05$). N2 and N3 dietary treatment groups increased the content of GSH, activity of CAT, and decreased the concentration of MDA in ovarian tissues ($P < 0.05$). Moreover, the N2 group enhanced the content of GSH, and the activity of CAT significantly compared with those in N1 groups ($P < 0.05$). No significant difference was found in the activity of CAT and the concentrations of GSH and MDA in ovarian tissues between C and N1, and N1 and N3 ($P > 0.05$, Table 5).

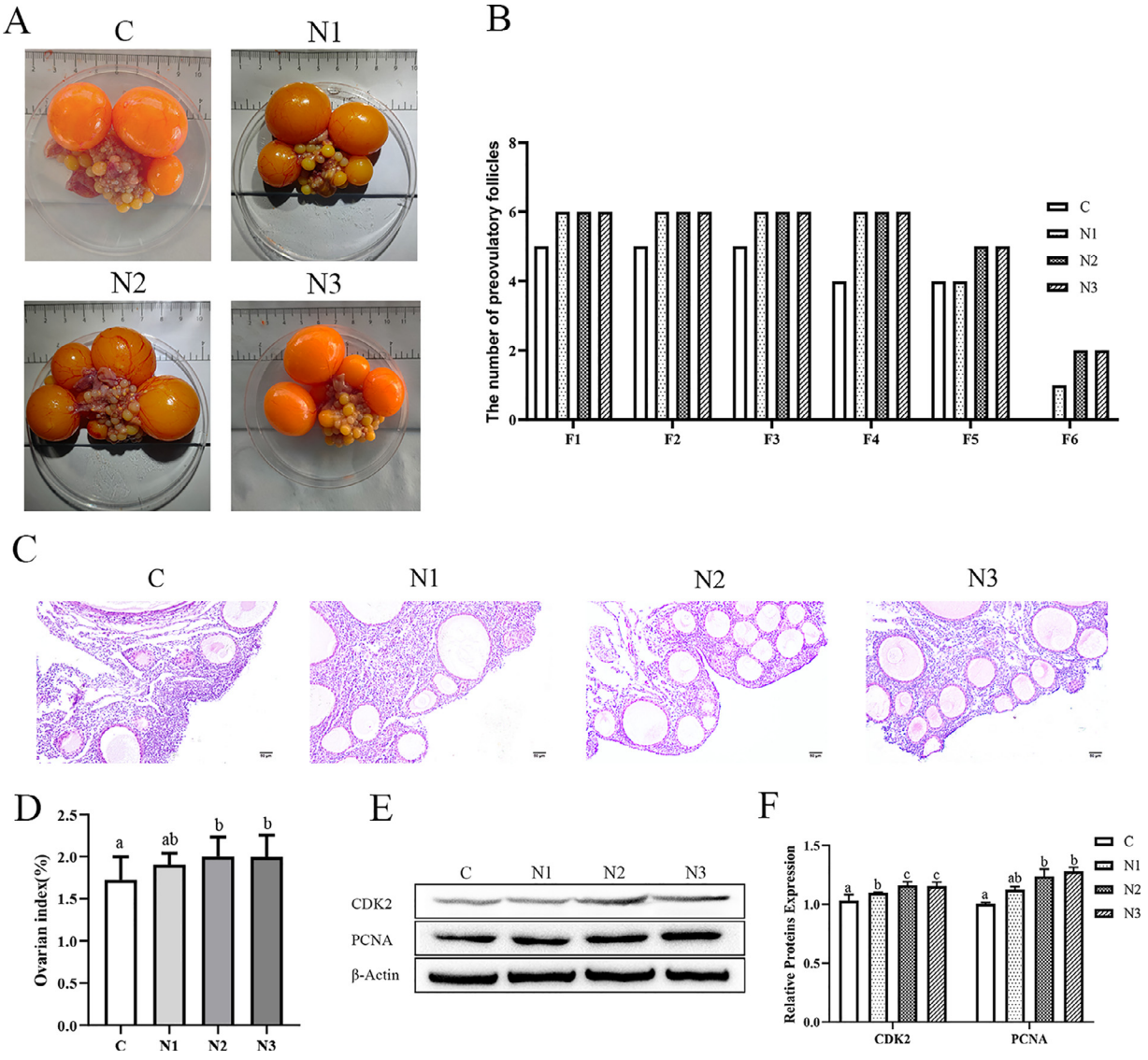

Figure 2. Ovarian morphology, index, and protein expression of Three-Yellow breeder hens. [a,b]Mean values with different superscripts letters denote statistical significantly difference ($P < 0.05$). (A) Ovary morphology in each group; (B) Numbers of the preovulatory follicles (with diameters over 12 mm) in each group (n = 12). (C) Ovarian histomorphology in each group, scale bar: 50 μm. (D) Ovarian index in each group (n = 12). (E) Western blot analysis of CDK2 and PCNA in ovary. F: Expression levels of proteins related to cell proliferation of ovarian tissues in each group. Abbreviations: C, basal diet group; N1, N2, and N3, basal diet supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively.

Table 5. Antioxidant parameters in ovarian tissues of hens.

Item	Groups				SEM	*P*-value		
	C	N1	N2	N3		ANOVA	Linear	Quadratic
GSH (μmol/gprot)	8.56[a]	8.98[ab]	9.54[c]	9.45[bc]	0.243	0.001	<0.001	0.149
CAT (U/mgprot)	15.29[a]	15.56[ab]	16.15[c]	16.05[bc]	0.265	0.006	0.001	0.328
T-SOD (U/mgprot)	21.27[a]	23.06[b]	23.44[b]	23.23[b]	0.632	0.005	0.003	0.030
T-AOC (U/mgprot)	0.882[a]	1.21[b]	1.23[b]	1.24[b]	0.043	<0.001	<0.001	<0.001
MDA (nmol/mgprot)	12.81[a]	11.84[ab]	11.05[b]	11.20[b]	0.543	0.009	0.002	0.149
H_2O_2 (mmol/gprot)	20.80[a]	18.64[b]	16.58[b]	16.81[b]	1.042	0.001	<0.001	0.113

[a-c]Mean values within a row with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 12). Abbreviations: C, basal diet group; CAT, catalase; GSH, glutathione; H_2O_2, Hydrogen peroxide; MDA, malondialdehyde; N1, N2, and N3, basal diets supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.

Ovarian Antioxidant Gene Expression

Compared with C group, expression levels of *CAT, SOD1, GPX1, GLRX*, and *PRDX3* in 3 treatment groups enhanced significantly ($P < 0.05$). Furthermore, the transcription levels of *GSR* in N2 and N3 groups were significantly higher than that in C group ($P < 0.05$; Figure 3). However, No significant difference was detected in the expression of *GSR* between C and N1 groups ($P = 0.248$). No significant difference was found among the 3 treatment groups ($P > 0.05$).

Serum Antioxidant Capacity

After feeding with naringin for 4 wk, activity of T-AOC and CAT in serum of N2 and N3 group were increased linearly than those in other 2 groups ($P < 0.05$). However, after 4 wk of feeding, dietary supplementation with naringin did not alter the activity of T-SOD, and the contents of GSH, MDA, and H_2O_2 in serum ($P > 0.05$, Table 6).

At the end of the experiment, dietary supplemented with naringin of 3 doses were linearly improved the activities of T-SOD and T-AOC in serum ($P < 0.05$), and the concentration of MDA in serum was a decreased linearly ($P < 0.05$). There was no significant difference in parameters mentioned above among 3 doses of treatments ($P > 0.05$). In N2 and N3 groups, the contents of H_2O_2 in serum were significantly lower than that in C group ($P < 0.05$). No significant difference was found between N1 group and C group ($P = 0.289$). Dietary supplemented with 0.2% naringin (N2) and 0.4% naringin (N3) both linearly improved the content of GSH and the activity of CAT in serum ($P < 0.05$), while no significant changes of them was observed in them between N1 and C groups ($P > 0.05$, Table 6).

DISCUSSION

During the late laying period, laying performance and egg quality of hens reduce dramatically (Lv et al., 2019). Recently, as antioxidants, many kinds of plant extracts are added to the diets in order to improve the egg production and prolong the laying lifespan of hens (Xie et al., 2019; Chen et al., 2021; Zhang et al., 2021). Naringin, as a natural flavonoid extract from plants, could benefit the health status of animals attributes to its properties of hormonal regulation, anti-oxidant, anti-inflammatory, antitoxicity, antineuroinflammation and metabolic regulation (Caglayan et al., 2018; Akintunde et al., 2020; Shirani et al., 2020).

Previous study on 1-day-old Ross broiler chickens showed that dietary addition of naringin (0.75 and 1.5 g/kg) for 42 d had no effect on the average body or carcass weight (Goliomytis et al., 2015). Similarly, Iskender et al. (2017) found that dietary supplemented with

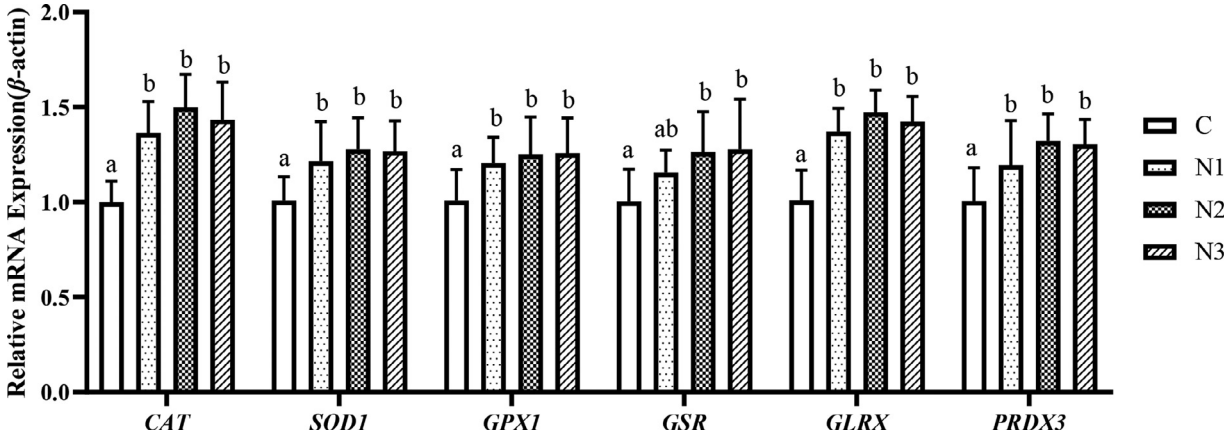

Figure 3. Transcription levels of antioxidant genes in ovarian tissues of hens. [a,b]Mean values with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 12). Abbreviations: C, basal diet group; N1, N2, and N3, basal diet supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively; *CAT*, catalase; *SOD1*, superoxide dismutase 1; *GPX1*, glutathione peroxidase 1; *GSR*, glutathione reductase; *GL*R, glutaredoxin; PRDX3, peroxiredoxin 3.

Table 6. Antioxidant parameters in serum of hens.

Item	Groups				SEM	*P*-value		
	C	N1	N2	N3		ANOVA	Linear	Quadratic
58 W								
GSH (μmol/L)	2.29	2.37	2.42	2.44	0.228	0.913	0.495	0.822
CAT (U/mL)	4.31[a]	4.64[b]	5.02[c]	5.04[c]	0.165	<0.001	<0.001	0.189
T-SOD (U/mL)	86.02	86.49	86.60	86.74	1.128	0.927	0.529	0.835
T-AOC (U/mL)	8.74[a]	9.28[ab]	9.96[b]	9.97[b]	0.422	0.013	0.002	0.374
MDA (nmol/mL)	12.94	12.46	12.14	12.04	0.622	0.457	0.131	0.673
H_2O_2 (mmol/L)	123.65	118.86	115.38	114.91	4.472	0.196	0.042	0.497
62 W								
GSH (μmol/L)	2.05[a]	2.13[a]	2.77[b]	2.74[b]	0.172	<0.001	<0.001	0.610
CAT (U/mL)	4.10[a]	4.39[ab]	4.75[b]	4.73[b]	0.175	0.001	<0.001	0.209
T-SOD (U/mL)	80.41[a]	85.01[b]	85.11[b]	85.77[b]	1.637	0.007	0.003	0.096
T-AOC (U/mL)	7.59[a]	8.88[b]	9.25[b]	9.31[b]	0.445	0.001	<0.001	0.057
MDA (nmol/mL)	16.17[a]	14.63[b]	13.16[c]	13.12[c]	0.654	<0.001	<0.001	0.112
H_2O_2 (mmol/L)	126.53[a]	118.25[ab]	110.06[b]	110.12[b]	4.608	0.002	<0.001	0.207

[a-c]Mean values within a row with different superscripts letters denote statistical significantly difference ($P < 0.05$, n = 12). Abbreviations: C, basal diet group; CAT, catalase; GSH, glutathione; H_2O_2, Hydrogen peroxide; MDA, malondialdehyde; N1, N2, and N3, basal diets supplemented with 0.1%, 0.2%, and 0.4% of naringin, respectively; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.

hesperidin (0.5 g/kg), naringin (0.5 g/kg), or quercetin (0.5 g/kg) did not affect the body weight of 28-wk-old Lohmann White laying hens. In line with these studies, our results showed that dietary addition of naringin (0.1%, 0.2%, and 0.4%) for 8 wk had no effect on body weight and appearance of Three-Yellow breeder hens during the late laying period. However, in our study, from the 4th wk of the feeding trials, the egg laying rate of N1, N2, and N3 groups were significantly higher than that in the C group. These results indicated that naringin as an additive to diet could improve the egg laying rate rather than enhancing the growth performance of Three-Yellow breeder hens in the late laying stage.

It is demonstrated that egg laying performance and egg quality could be influenced by dietary supplemented with additives extracted from plants (Xie et al., 2019). Wang et al. (2018) demonstrated that supplementing tea polyphenols or tea catechins (200 mg/kg) in diet for 10 wk improved the laying rate, egg mass, and feed conversion rate of 64-wk-old Hy-Line Brown laying hens. Amevor et al. (2021b) reported that the feed intake, laying rate and egg weight increased after dietary supplemented with quercetin (0.4 g/kg) for 10 wk in 52-wk-old Tianfu breeder hens. Study of Lv et al. (2019) showed that diet addition of genistein (40 and 400 mg/kg) improved egg laying rate significantly of 55-wk-old Ross 308 brooder hens. Consistent with these studies, we observed that naringin supplementation (0.1%, 0.2%, and 0.4%) in the feed of 54-wk-old Three-Yellow breeder hens increased the laying rate, egg mass, and feed egg ratio. Interestingly, a previous study reported that supplementing with naringin (0.75 and 1.5 g/kg) in 54-wk-old Lohmann Brown laying hens diet for 67 d had no effect on laying performance (Goliomytis et al., 2019). We speculate these differences may be due to the different varieties and laying stages of hens, and dissimilar doses of naringin used in the trials.

As great factors affecting hatching rate of breeding eggs, the quality and breakage rate of breeding eggs are closely related to eggshell strength and thickness. In hens, increased breakage rates caused by decreasing eggshell strength during the aging process result in declines in reproductive performance and commercial value (Qiu et al., 2020; Amevor et al., 2021b). Previous studies indicated that dietary flavonoids supplementation in hens had specific effects on egg quality. Liu et al. (2013) suggested that supplementing quercetin (0.2 and 0.4 g/kg) in diet of Hessian laying hens for 8 wk increased Haugh unit, eggshell strength, and eggshell thickness. Study of Liu et al. (2014) suggested that egg quality was not influenced by quercetin (0.2, 0.4, and 0.6 g/kg) supplementation. Similarly, Golimoytis et al. (2019) found that hesperidin or naringin (0.75 and 1.5 g/kg) supplementation had no effects on egg quality except yolk color of Lohmann Brown laying hens. In our present study, after 8 wk of dietary naringin supplementation, the parameters of egg quality did not change significantly, except egg yolk color. These results are in line with the results of a previous report that enhanced color of egg yolk caused by increased activity of antioxidant enzyme by neohesperidin dihydrochalcone additive in the Lohmann laying hens (Zhu et al., 2021). This may be related to the naringin and neohesperidin are both flavonoids that could increase the oxidation capacity of hens to prevent lutein from being oxidized and increase pigment deposition.

In hens, normal ovarian function plays a crucial role in the maintenance of laying performance. Ovarian index could partially reflect the reproductive function (Han et al., 2020). And the number of ovarian follicles determines the laying rate of the hen (Hao et al., 2020). Long et al. (2017) reported that dietary supplemented with octacosanol improved the laying rate of Hy-Line Brown laying hens by increasing the ovarian weight and the number of follicles in the ovary. In hens, high laying rate is always accompanied with high cell proliferation and low follicular atresia rate (Wang et al., 2021c). The research of Dai et al. (2021) on Qiling breeder hens suggested that dietary addition with hawthorn-leaves flavonoids (30 and 60 mg/kg) improved ovary relative weight and the laying rate. Previous study reported that ovarian granulosa cells apoptosis induced by oxidative stress

with aging resulted in ovarian follicle atresia (Amevor et al., 2021a). In this study, naringin supplementation at 0.2% and 0.4% both increased the number of preovulatory and growing follicles, ovarian index, and ovarian somatic cell proliferation of the Three-Yellow breeder hens at the late laying stage. We speculated that naringin could improve the antioxidant capacity of Three-Yellow breeder hens, thereby promoting ovarian somatic cell proliferation and alleviating the follicular atresic caused by oxidative stress. However, whether the results are consistent with our hypothesis needs further study.

Oxidative stress induced by the disruption of balance between oxidants and the antioxidant system could damage physiological functions of the ovary and result in reproductive failure (Xie et al., 2019). Studies in rats have shown that naringin could prevent clophosphamide-induced hepatotoxicity by increasing the activity of CAT, GSR, and GSH-Px, and the content of GSH in liver tissues (Akamo et al., 2021). Naringin have been reported to reduce MDA concentration in lamb carcass and prolong meat storage (Simitzis et al., 2019). In laying hens dietary supplemented with neohesperidin (flavonoids) increased the antioxidant capacity by enhancing T-AOC of the serum (Zhu et al., 2021). Furthermore, flavonoids could up-regulate the expression of antioxidant genes. Boots et al. (2020) found that quercetin could improve the transcription levels of *SOD2, CAT,* and *HO-1* in mice. Wan et al. (2020) demonstrated that hesperetin inhibited oxidative stress and attenuated acetaminophen-induced hepatotoxicity via the up-regulation of *HO-1* expression. Another study in broilers suggested that naringin up-regulated the expression of antioxidant genes *GSR, ACOX1,* and *PPARA* in the liver tissues (Hager-Theodorides et al., 2021). In this study, dietary supplemented with 0.2% and 0.4% naringin could improve the antioxidant capacity of the hens by enhanced the concentration of GSH, the activities CAT, T-AOC and T-SOD, and decreased the concentrations of MDA and H_2O_2 in the ovarian tissues and serum, as well as increased transcription levels of antioxidant genes were observed. Our results indicated that naringin supplementation improved the antioxidant capacity of the ovary and the serum of the hens.

In conclusion, this study provides the evidence that dietary supplemented with naringin has positive effects on laying rate, egg mass, feed egg ratio, and yolk color of hens, as well as the antioxidant capacity of ovary and the serum of Three-Yellow breeder hens during the late laying period. Taken together, a level of 0.2% or 0.4% naringin addition in the diet is more helpful than 0.1% for improving the laying performance of Three-Yellow breeder hens at the late laying stage.

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DISCLOSURES

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

Abbas, A. O., A. A. Alaqil, H. S. El-Beltagi, H. K. Abd El-Atty, and N. N. Kamel. 2020. Modulating laying hens productivity and immune performance in response to oxidative stress induced by E. coli challenge using dietary propolis supplementation. Antioxidants 9:893.

Akamo, A. J., S. O. Rotimi, D. I. Akinloye, R. N. Ugbaja, O. O. Adeleye, O. A. Dosumu, O. E. Eteng, G. Amah, A. Obijeku, and O. E. Cole. 2021. Naringin prevents cyclophosphamide-induced hepatotoxicity in rats by attenuating oxidative stress, fibrosis, and inflammation. Food Chem. Toxicol. 153:112266.

Akintunde, J. K., T. E. Akintola, G. O. Adenuga, Z. A. Odugbemi, R. O. Adetoye, and O. G. Akintunde. 2020. Naringin attenuates Bisphenol-A mediated neurotoxicity in hypertensive rats by abrogation of cerebral nucleotide depletion, oxidative damage and neuroinflammation. Neurotoxicology 81:18–33.

Alvarez, R., and P. M. Hocking. 2012. Changes in ovarian function and egg production in commercial broiler breeders through 40 weeks of lay. Br. Poult. Sci. 53:386–393.

Amevor, F. K., Z. Cui, X. Du, Z. Ning, G. Shu, N. Jin, X. Deng, Y. Tian, Z. Zhang, X. Kang, D. Xu, G. You, Y. Zhang, D. Li, Y. Wang, Q. Zhu, and X. Zhao. 2021a. Combination of quercetin and vitamin E supplementation promotes yolk precursor synthesis and follicle development in aging breeder hens via liver-blood-ovary signal axis. Animals (Basel) 11:1915.

Amevor, F. K., Z. Cui, Z. Ning, X. Du, N. Jin, G. Shu, X. Deng, Q. Zhu, Y. Tian, D. Li, Y. Wang, Z. Zhang, and X. Zhao. 2021b. Synergistic effects of quercetin and vitamin E on egg production, egg quality, and immunity in aging breeder hens. Poult. Sci 100:101481.

An, B. K., W. D. Choo, C. W. Kang, J. Lee, and K. W. Lee. 2019. Effects of dietary lycopene or tomato paste on laying performance and serum lipids in laying hens and on malondialdehyde content in egg yolk upon storage. J. Poult. Sci. 56:52–57.

Boots, A. W., C. Veith, C. Albrecht, R. Bartholome, M. J. Drittij, S. Claessen, A. Bast, M. Rosenbruch, L. Jonkers, F. J. van Schooten, and R. Schins. 2020. The dietary antioxidant quercetin reduces hallmarks of bleomycin-induced lung fibrogenesis in mice. BMC Pulm. Med. 20:112.

Caglayan, C., Y. Temel, F. M. Kandemir, S. Yildirim, and S. Kucukler. 2018. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ. Sci. Pollut. Res. Int. 25:20968–20984.

Chen, F., H. Zhang, E. Du, F. Jin, C. Zheng, Q. Fan, N. Zhao, W. Guo, W. Zhang, S. Huang, and J. Wei. 2021. Effects of Magnolol on egg production, egg quality, antioxidant capacity, and intestinal health of laying hens in the late phase of the laying cycle. Poult. Sci. 100:835–843.

Chtourou, Y., B. Aouey, S. Aroui, M. Kebieche, and H. Fetoui. 2016. Anti-apoptotic and anti-inflammatory effects of naringin on cisplatin-induced renal injury in the rat. Chem. Biol. Interact. 243:1–9.

Dai, H., Z. Lv, C. Hu, Z. Shi, X. Wei, S. Jin, Y. Yuan, D. Yu, and F. Shi. 2020. Alpha-lipoic acid improves the reproduction performance of breeder hens during the late egg-laying period. J. Anim. Physiol. An. N. 104:1788–1797.

Dai, H., Z. Lv, Z. Huang, N. Ye, S. Li, J. Jiang, Y. Cheng, and F. Shi. 2021. Dietary hawthorn-leaves flavonoids improves ovarian function and liver lipid metabolism in aged breeder hens. Poult. Sci. 100:101499.

Goliomytis, M., N. Kartsonas, M. A. Charismiadou, G. K. Symeon, P. E. Simitzis, and S. G. Deligeorgis. 2015. The influence of naringin or hesperidin dietary supplementation on broiler meat quality and oxidative stability. PLoS One 10:e141652.

Goliomytis, M., P. Simitzis, A. Papalexi, N. Veneti, A. L. Hager-Theodorides, M. A. Charismiadou, and S. G. Deligeorgis. 2019. Influence of citrus flavonoids on laying hen performance, inflammatory immune response, egg quality and yolk oxidative stability. Br. Poult. Sci. 60:272–278.

Hager-Theodorides, A. L., T. Massouras, P. E. Simitzis, K. Moschou, E. Zoidis, E. Sfakianaki, K. Politi, M. Charismiadou, M. Goliomytis, and S. Deligeorgis. 2021. Hesperidin and naringin improve broiler meat fatty acid profile and modulate the expression of genes involved in fatty acid beta-oxidation and antioxidant defense in a dose-dependent manner. Foods 10:739.

Han, X., L. Bai, Y. Wang, Y. Li, D. Zhao, G. Hu, J. Hao, M. Gu, X. Guo, and W. Wang. 2020. Ovarian index of km mice influenced by longer term consumption of microwave-heated milk. J. Food Prot. 83:1066–1071.

Hao, E. Y., H. Chen, D. H. Wang, C. X. Huang, Y. G. Tong, Y. F. Chen, R. Y. Zhou, and R. L. Huang. 2020. Melatonin regulates the ovarian function and enhances follicle growth in aging laying hens via activating the mammalian target of rapamycin pathway. Poult. Sci. 99:2185–2195.

Huang, Y., Z. Ma, X. Kuang, Q. Zhang, H. Li, and D. Lai. 2021b. Sodium alginate-bioglass-encapsulated hAECs restore ovarian function in premature ovarian failure by stimulating angiogenic factor secretion. Stem Cell Res. Ther. 12:223.

Huang Z., Lv Z., Dai H., Li S., Jiang J., Ye N., Zhu S., Wei Q. and Shi F. 2021a. Dietary mulberry-leaf flavonoids supplementation improves liver lipid metabolism and ovarian function of aged breeder hens [e-pub ahead of print]. J. Anim. Physiol Anim Nutr (Berl). Accessed July 2022. doi:10.1111/ /jpn.13658.

Iskender, H., G. Yenice, E. Dokumacioglu, O. Kaynar, A. Hayirli, and A. Kaya. 2017. Comparison of the effects of dietary supplementation of flavonoids on laying hen performance, egg quality and egg nutrient profile. Br. Poult. Sci. 58:550–556.

Jiang, X., A. Li, Y. Wang, M. Iqbal, M. Waqas, H. Yang, Z. Li, K. Mehmood, H. Qamar, and J. Li. 2020. Ameliorative effect of naringin against thiram-induced tibial dyschondroplasia in broiler chicken. Environ. Sci. Pollut. Res. Int. 27:11337–11348.

Kim, J. K., J. H. Park, H. J. Ku, S. H. Kim, Y. J. Lim, J. W. Park, and J. H. Lee. 2018. Naringin protects acrolein-induced pulmonary injuries through modulating apoptotic signaling and inflammation signaling pathways in mice. J. Nutr. Biochem. 59:10–16.

Li, G. M., L. P. Liu, B. Yin, Y. Y. Liu, W. W. Dong, S. Gong, J. Zhang, and J. H. Tan. 2020. Heat stress decreases egg production of laying hens by inducing apoptosis of follicular cells via activating the FasL/Fas and TNF-alpha systems. Poult. Sci. 99:6084–6093.

Lim, Y. J., J. H. Kim, J. H. Pan, J. K. Kim, T. S. Park, Y. J. Kim, J. H. Lee, and J. H. Kim. 2018. Naringin protects pancreatic beta-cells against oxidative stress-induced apoptosis by inhibiting both intrinsic and extrinsic pathways in insulin-deficient diabetic mice. Mol. Nutr. Food Res. 62:10.

Liu, H. N., Y. Liu, L. L. Hu, Y. L. Suo, L. Zhang, F. Jin, X. A. Feng, N. Teng, and Y. Li. 2014. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poult. Sci. 93:347–353.

Liu, Y., Y. Li, H. N. Liu, Y. L. Suo, L. L. Hu, X. A. Feng, L. Zhang, and F. Jin. 2013. Effect of quercetin on performance and egg quality during the late laying period of hens. Br. Poult. Sci. 54:510–514.

Long, L., S. G. Wu, F. Yuan, H. J. Zhang, J. Wang, and G. H. Qi. 2017. Effects of dietary octacosanol supplementation on laying performance, egg quality, serum hormone levels, and expression of genes related to the reproductive axis in laying hens. Poult. Sci. 96:894–903.

Lv, Z. P., S. J. Yan, G. Li, D. Liu, and Y. M. Guo. 2019. Genistein improves the reproductive performance and bone status of breeder hens during the late egg-laying period. Poult. Sci. 98:7022–7029.

May-Panloup, P., L. Boucret, J. Chao De La Barca, V. Desquiret-Dumas, V. Ferré-L'Hotellier, C. Morinière, P. Descamps, V. Procaccio, and P. Reynier. 2016. Ovarian ageing: the role of mitochondria in oocytes and follicles. Hum. Reprod. Update 22:725–743.

Qiu, J. L., Q. Zhou, J. M. Zhu, X. T. Lu, B. Liu, D. Y. Yu, G. Lin, T. Ao, and J. M. Xu. 2020. Organic trace minerals improve eggshell quality by improving the eggshell ultrastructure of laying hens during the late laying period. Poult. Sci. 99:1483–1490.

Ren, X., R. Li, J. Liu, K. Huang, S. Wu, Y. Li, and C. Li. 2018. Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses. Environ. Pollut. 243:833–841.

Sharma, D., G. K. Sangha, and K. S. Khera. 2015. Triazophos-induced oxidative stress and histomorphological changes in ovary of female Wistar rats. Pestic Biochem. Physiol. 117:9–18.

Shirani, K., B. S. Yousefsani, M. Shirani, and G. Karimi. 2020. Protective effects of naringin against drugs and chemical toxins induced hepatotoxicity: a review. Phytother. Res. 34:1734–1744.

Simitzis, P. E., M. A. Charismiadou, M. Goliomytis, A. Charalambous, I. Ntetska, E. Giamouri, and S. G. Deligeorgis. 2019. Antioxidant status, meat oxidative stability and quality characteristics of lambs fed with hesperidin, naringin or alpha-tocopheryl acetate supplemented diets. J. Sci. Food Agric. 99:343–349.

Wan, J., G. Kuang, L. Zhang, R. Jiang, Y. Chen, Z. He, and D. Ye. 2020. Hesperetin attenuated acetaminophen-induced hepatotoxicity by inhibiting hepatocyte necrosis and apoptosis, oxidative stress and inflammatory response via upregulation of heme oxygenase-1 expression. Int. Immunopharmacol. 83:106435.

Wang, J., R. Jia, H. Gong, P. Celi, Y. Zhuo, X. Ding, S. Bai, Q. Zeng, H. Yin, S. Xu, J. Liu, X. Mao, and K. Zhang. 2021a. The effect of oxidative stress on the chicken ovary: involvement of microbiota and melatonin interventions. Antioxidants (Basel) 10:1422.

Wang, J., C. Zhang, S. Zhao, X. Ding, S. Bai, Q. Zeng, K. Zhang, Y. Zhuo, S. Xu, X. Mao, H. Peng, and Z. Shan. 2021c. Dietary apple pectic oligosaccharide improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders. Poult. Sci. 100:100976.

Wang, J., H. J. Zhang, L. Xu, C. Long, K. G. Samuel, H. Y. Yue, L. L. Sun, S. G. Wu, and G. H. Qi. 2016. Dietary supplementation of pyrroloquinoline quinone disodium protects against oxidative stress and liver damage in laying hens fed an oxidized sunflower oil-added diet. Animal 10:1129–1136.

Wang, R., G. Wu, T. Dai, Y. Lang, Z. Chi, S. Yang, and D. Dong. 2021b. Naringin attenuates renal interstitial fibrosis by regulating the TGF-beta/Smad signaling pathway and inflammation. Exp. Ther. Med. 21:66.

Wang, X. C., X. H. Wang, J. Wang, H. Wang, H. J. Zhang, S. G. Wu, and G. H. Qi. 2018. Dietary tea polyphenol supplementation improved egg production performance, albumen quality, and magnum morphology of Hy-Line Brown hens during the late laying period. J. Anim. Sci. 96:225–235.

Wu, H., H. Li, Y. Hou, L. Huang, J. Hu, Y. Lu, and X. Liu. 2022. Differences in egg yolk precursor formation of Guangxi Ma chickens with dissimilar laying rate at the same or various ages. Theriogenology 184:13–25.

Wu, Y., Y. Li, X. Liao, Z. Wang, R. Li, S. Zou, T. Jiang, B. Zheng, P. Duan, and J. Xiao. 2017. Diabetes induces abnormal ovarian function via triggering apoptosis of granulosa cells and suppressing ovarian angiogenesis. Int. J. Biol. Sci. 13:1297–1308.

Xie, T., S. P. Bai, K. Y. Zhang, X. M. Ding, J. P. Wang, Q. F. Zeng, H. W. Peng, H. Y. Lu, J. Bai, Y. Xuan, and Z. W. Su. 2019. Effects of lonicera confusa and astragali radix extracts supplementation on egg production performance, egg quality, sensory evaluation, and antioxidative parameters of laying hens during the late laying period. Poult. Sci. 98:4838–4847.

Xu, G. F., and K. W. Chen. 2003. Photograph album of China indigenous poultry breeds (First Edition). Pages 114-115 in Guangxi Sanhuang. China Agriculture Press, Beijing, China.

Yao, H., Y. Hu, Q. Wang, Y. Zhang, K. Rao, and S. Shi. 2022. Effects of dietary dimethylglycine supplementation on laying performance, egg quality, and tissue index of hens during late laying period. Poult. Sci. 101:101610.

Yu, H., M. Kuang, Y. Wang, S. Rodeni, Q. Wei, W. Wang, and D. Mao. 2019. Sodium arsenite injection induces ovarian oxidative stress and affects steroidogenesis in rats. Biol. Trace Elem. Res. 189:186–193.

Yuan, Z. H., K. Y. Zhang, X. M. Ding, Y. H. Luo, S. P. Bai, Q. F. Zeng, and J. P. Wang. 2016. Effect of tea polyphenols on production performance, egg quality, and hepatic antioxidant status of laying hens in vanadium-containing diets. Poult. Sci. 95:1709–1717.

Zeng, X., W. Su, Y. Zheng, Y. He, Y. He, H. Rao, W. Peng, and H. Yao. 2019. Pharmacokinetics, tissue distribution, metabolism, and excretion of naringin in aged rats. Front. Pharmacol. 10:34.

Zhang, L., G. Zhong, W. Gu, N. Yin, L. Chen, and S. Shi. 2021. Dietary supplementation with daidzein and Chinese herbs, independently and combined, improves laying performance, egg quality and plasma hormone levels of post-peak laying hens. Poult. Sci. 100:101115.

Zhu, A. N., K. Y. Zhang, J. P. Wang, S. P. Bai, Q. F. Zeng, H. W. Peng, and X. M. Ding. 2021. Effect of different concentrations of neohesperidin dihydrochalcone on performance, egg quality, serum biochemistry and intestinal morphology in laying hens. Poult. Sci. 100:10 1097.