Effects of capsaicin on laying performance, follicle development, and ovarian antioxidant capacity in aged laying ducks

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ABSTRACT The present study was conducted to evaluate the effects of dietary addition of capsaicin (**CAP**) on egg production performance, follicular development, and ovarian antioxidant capacity in laying ducks. Three hundred seventy eight 58-wk-old laying ducks were randomly divided into 3 treatments, each treatment consisted 6 replicates, with 12 individually caged laying ducks per replicate. Ducks fed a basal diet served as control, the other 2 groups of ducks were fed the same diet containing 150 mg/kg CAP but in the manner of feed restriction (pair-fed) or ad libitum fed. The experiment lasted for 8 wk. The results showed that the dietary supplementation with CAP under conditions of ad libitum feeding increased feed intake ($P < 0.001$) and tended ($P < 0.1$) to increase egg production and egg weight in laying ducks but had no effects on daily egg mass and feed conversion ratio. The relative weight of large yellow follicles from the 2 CAP-supplemented groups at 64 wk of age were significantly higher than that of the controls ($P = 0.01$). The relative weight of the small yellow follicles in the CAP free-fed group was significantly higher than that of the other 2 groups ($P < 0.01$). Capsaicin supplementation under ad libitum feding conditions tended to increase the number of dominant follicles in laying ducks ($P = 0.06$). The ovarian mRNA expression of genes related to calcium signaling (*TRPV4*, *ATP2A2*, *ITPR1*, and *CaM*) in the CAP ad libitum fed groups were significantly higher than those of the other 2 groups ($P < 0.05$). The ovarian mRNA expression of *CDK1* in CAP free-fed ducks was significantly higher than that of the other 2 groups ($P = 0.01$). Capsaicin supplementation significantly increased the plasma glutathione peroxidase activity ($P < 0.01$) in comparison with the control group but reduced the malondialdehyde content in the ovaries of laying ducks ($P < 0.01$). The results of this study indicates that dietary supplementation of CAP increased feed intake and improved egg production performance probably by activating calcium signaling pathway and improving redox status.

Key words: capsaicin, laying duck, follicle, antioxidant

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INTRODUCTION

Capsaicin (8-methyl-N-vanilla base-6-nonene amide) (**CAP**) is the active component of chili peppers and is extremely spicy vanilla amide alkaloid, which mainly exists in the mature red pepper; it accounts for about 0.1 ~ 0.2% of the dry weight of the red pepper (Govindarajan and Sathyanarayana, 1991). Capsaicin excites sensory neurons by binding to its receptor in the plasma membrane and activating ligand-gated, nonselective cation channels (Nagy et al., 2004; Nakagawa and Hiura, 2006). Ovarian functions and folliculogenesis develop in response to the central nervous system through the release of gonadotropin-releasing hormone, which in turn stimulates the production and release of follicle-stimulating hormone and luteinizing hormone in turn from the pituitary (Jeong and Kaise, 2006). Some researchers have suggested that CAP-sensitive sensory nerves could play a role in regulating

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the fertility and follicle development in female rats (Traurig et al., 1984; Pintado et al., 2003).

The effects of CAP on the female reproductive system, however, are contradictory. Alatriste et al. (2013) reported that high-dose CAP administration caused sensory denervation leading to poor ovarian follicular development and a delay in the onset of puberty of guinea pigs. Similarly, Pintado et al. (2003) found that female rats neonatally treated with a high dose of CAP (50 mg/kg) exhibited an apparently normal courtship behavior but a lower reproductive success and litter size, compared with control. However, low-dose CAP was reported to protect the follicles from apoptosis and atresia and stimulate follicular development (Zik et al., 2010a,b). Ozer et al. (2005) found in laying hens that dietary supplementation with red hot pepper (10 g/kg diet) improved follicular development and laying performance. Furthermore, it is reported that 24- and 48-h administrations of low-dose CAP induced proliferation in rat granulosa cells (Güler and Zik, 2018), which indicates that low-dose and short-term administration of CAP may have a positive effect on ovarian folliculogenesis by increasing the proliferation of granulosa cells. Therefore, it was indicated based on these studies that high-dose CAP treatments had a neurotoxic effect, while low-dose CAP was found to have positive effects on the ovary.

Until date, the effects of CAP on follicle maturation in aged laying birds remain unclear. This study, therefore, aimed to determine the effects of dietary supplementation with CAP on follicular development, egg laying performance, and ovarian antioxidant capacity. This study is expected to contribute a scientific basis for the development of CAP as a green feed additive for poultry.

MATERIALS AND METHODS

Animals and Management

All animal care procedures in this study followed the guideline of Institutional Animal Care and Use Committee of Institute of Animal Science, Guangdong Academy of Agricultural Sciences. A total of 378 female Longyan laying ducks with the same genetic background and comparable BW (1.21 ± 0.25 kg) at 58 wk of age were allotted randomly into 3 treatments, with 6 replicates of 21 ducks each. Ducks were housed in individual galvanized battery cages (length 27.8 cm × width 40 cm × height 55 cm). Ducks that were fed a corn–soybean–based diet served as controls, the other 2 groups of ducks were fed corn–soybean–basal diets supplemented with 150 mg/kg CAP commercial product (Guangzhou Leader Bio-Technology Co., Ltd.), in the manner of ad libitum (**AF**) or pair-fed (**PF**). The commercial CAP product contains 98% diluent (stearic acid) and 2% CAP; of which, CAP and dihydrocapsaicin consist of 91% of total CAP, as determined by the method of Othman et al. (2011). CAP (150 mg/kg diet) were supplemented in place of zeolite powder in the premix. Diets for each treatment were prepared individually by mixing and then pelleting feed ingredients and experimental diets were provided in the form of pellet (3-mm diameter). Ducks that were PF were provided the same amount of diets as control ducks to minimize the potential effect of increased feed intake by CAP on the indicators. Experimental diets were prepared to meet the nutrient recommendation of laying ducks established by this laboratory (Table 1). Ducks had free access to water throughout and were subjected to 16L:8D per day. The experiment lasted for 8 wk.

Feed refusals were collected and weighed daily to determine feed intake on a replicate basis. To avoid feed scattering, the amount of offered feed in the control fed ducks was increased by 10 g/bird per day than that of the previous day if there were no feed refusal. Experimental diets supplemented with CAP (150 mg/kg diet) were provided for AF or PF ducks. For PF ducks, the diets were offered the same amount as that of the pervious day from basal control ducks. Egg number and egg weight were recorded daily on a replicate basis, and the average egg production rate, feed intake, average egg weight, egg mass, and feed conversion ratio were calculated for the whole experimental period (8 wk).

Sample Collection

At the end of the experiment, all ducks were fasted for 12 h, 2 randomly selected ducks from each replicate were weighed, 10 mL of blood was collected from the wing vein of ducks into vacuum blood collection tubes containing an anticoagulant and centrifuged at 3,000 × *g* for 15 min, and then the supernatant was collected and stored at −80°C. After blood sampling, ducks were killed by cervical dislocation for tissue collection. The ovaries were collected and weighed to calculate the ovarian index [(ovary weight g/BW g) × 100]. Large yellow follicles (diameter > 8 mm) were counted and weighed; the number of small yellow follicles (3 mm < diameter < 8 mm) and atresic small yellow follicles was recorded. The relative weights of total small yellow follicles and large yellow follicles to the ovarian weight were calculated (%). Ovarian tissue samples with removal of yellow follicles were collected and snap-frozen in liquid nitrogen and then stored at −80°C until analysis.

Total RNA was extracted from the ovaries using TRIzol (Invitrogen, Carlsbad, CA). The purity of RNA sample was detected by a nucleic acid quantifier (NanoDrop-2000; Thermo Fisher Scientific, Waltham, MA) by OD 260/280. RNA integrity was examined by electrophoresis with 1.0% agarose gel for 20 min. cDNA was synthesized by reverse transcription from 2.0 μg of high-quality RNA in a final volume of 30 μL as per the manufacturer's instructions (Takara, Otsu, Japan). After digestion and purification by DNase I (Takara, Otsu, Japan), RNA was reverse transcribed into cDNA by M-MLV reverse transcriptase (Promega, Madison, WI), and the cDNA samples were stored at −80°C.

Table 1. Composition and analysis of experimental diets (%, as fed).

Ingredients	%	Calculated nutrient and energy composition	
Corn	52.4	AME, MJ/kg	10.45
Soybean meal	26.0	CP, %	18.0
Wheat bran	10.2	Calcium, %	3.6
Limestone	8.64	Available phosphorus, %	0.35
Dicalcium phosphate	1.31	Lysine, %	0.95
Salt	0.30	Methionine, %	0.40
DL-Methionine	0.15	Methionine + cysteine, %	0.70
Premix[1]	1.00		
Total	100		

[1]The premix provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 1,800 IU; vitamin E, 26 IU; vitamin K_3, 1.0 mg; vitamin B_1, 3.0 mg; vitamin B_2, 9.6 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 0.03 mg; choline, 500 mg; D-calcium pantothenate, 28.5 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Fe, 50 mg; Cu, 10 mg; Mn, 90 mg; Zn, 90 mg; I, 0.50 mg, Se, 0.30 mg.

The mRNA expression of target genes were determined by real-time fluorescent quantitative PCR with an iQ5 CFX96 gene quantifier (Bio-Rad, Hercules, CA). PCR reaction system consisted of 10 μL SYBR Green PCR master Mix (Takara, Otsu, Japan), 0.5 μL upstream primer, 0.5 μL downstream primer, 1 μL cDNA template, and 8 μL water. The primers were designed by the software Premer Premier 6.0 and synthesized by Shanghai Biotechnology Engineering Co., Ltd. The primer sequences and parameters are shown in Table 2. Target gene expression was standardized to *β-actin* mRNA, using the **ΔCt** method as described by Chen et al. (2015b).

Assay of Plasma and Ovarian Antioxidant Indices

A quantity of 100 mg ovarian sample was homogenized in 9 mL of homogenization medium, mechanically homogenized under ice water bath conditions, centrifuged at 2,500 × *g* for 10 min at 4°C, and the supernatant liquid was collected for biochemical analysis. The plasma and ovarian enzymatic activity of total superoxide dismutase and glutathione peroxidase, as well as malondialdehyde (**MDA**) content and total antioxidant capacity, were determined using a commercial kit purchased from Nanjing Jiancheng Bioengineering Research

Table 2. Primer sequences used for quantitative real-time PCR.

Genes[1]	Accession number	Primer sequence(5′-3′)	Product length (bp)
TRPV4	XM_032199198.1	F : TCATCACCCTTCTCACCG R : CACAATCACCAGCACCGA	143
CALB1	XM_013108520.2	F : AAGAAGGCAGGCTTGGACT R : GGCACCTAAAGAACAACAGG	161
ATP2A2	XM_021270084.2	F : TTAATGAGGATGCCCCCGTG R : ACTCCAGTATTGCAGGTTCCA	232
Oral1	XM_027469677.1	F : CGCCATGTCTATCTCAGGGC R : TGCACTTCCACCATAGCCAC	138
ITPR1	XM_021272729.2	F : TGACCAGAATAAAAGAGACCCG R : TCGCCAGTTCATTGCAGTCT	242
CaM	D83350.1	F : CGAGGAAGAAATCCGTGAG R : TGACTTGCCCATCCCCAT	167
CDK1	XM_013095817.1	F : AGCCACTTTTCCATGGGGAC R : CAGGCCACCAGGTTTCCATT	146
CCNB2	XM_005025431.2	F : AGTCGGTACGCCCACATTAC R : GCGCTGTTACACCTACCAAC	201
Bcl2	XM_005028719.1	F : ACCTGGTTCTGAATAAGTGGGAT R : GGTTGTCTTCTCAGTGTTGCCT	187
Caspase3	XM_005030494.1	F : TGTTGAGGCAGACAGTGGACC R : GGAGTAATAGCCTGGAGCAGTAGA	100
FAS	XM_027459847.2	F: AGAACACAAAATGCGCCTGT R: TATGACCACAGCTGCAATGC	179
FOXL2	XM_027463986.1	F : CTGCGAGGACATGTTCGAGA R : TGACGTTCCCACCAGACATC	236
STAR	XM_027443533.1	F : ATGGCCAGGTCTGGGTCTG R : GCCTTAAATACGCCCGCTGA	188
CYP19A1	XM_021277353.2	F : CAAGAGGAGAACACAGCAAAGC R : TGTGAAATGAGGGGGCCAAT	221
β-actin	EF667345.1	F : GCTATGTCGCCCTGGATTT R : GGATGCCACAGGACTCCATAC	174

Abbreviations: ATP2A2, ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2; Bcl2, BCL2 Apoptosis Regulator; CALB1, Calbindin 1; CAM, Calmodulin; CCNB2, Cyclin B2; CDK1, cyclin dependent kinase 1; CYP19A1, Cytochrome P450 family 19 subfamily A member 1; FAS, Fas cell surface death receptor; FOXL2, Forkhead Box L2; ITPR1, Inositol 1,4,5-Trisphosphate Receptor Type 1; STAR, steroidogenic acute regulatory protein; TRPV4, the transient receptor potential vanilloid subfamily 4.

Institute (Nanjing, China). The concentrations of estradiol and progesterone were determined by radioimmunoassay using a commercial kit (Beijing North Institute of Biotechnology Co., Ltd., Beijing, China).

Statistical Analysis

The experimental data were analyzed by one-way GLM using the GLM procedure in SAS (SAS 9.0, SAS Institute Inc.). The multiple comparative analysis of Student–Newman–Keuls mean value was carried out when the ANOVA showed significant differences, and $P < 0.05$ was a significant difference.

RESULTS

As shown in Table 3, ducks that were AF fed diets with supplementation of CAP had higher daily feed intake than the control group ($P < 0.01$), while egg mass and feed conversion ration were not affected, but there was a tendency to increase the egg production rate of laying ducks ($P = 0.06$) and egg weight ($P = 0.08$) when ducks were AF fed with CAP-supplemented diets. However, supplementation of CAP in the manner of PF had no effects on the egg production performance, including egg production, egg weight, egg mass, and feed conversion ratio (**FCR**).

As shown in Table 4, the relative weight of large and small yellow follicles from both of the 2 CAP-supplemented groups were significantly higher than that of the controls ($P = 0.01$). The ovarian indices, except for small yellow follicle weight/ovarian weight, were not different between the 2 CAP-supplemented treatments ($P > 0.05$). The relative weight of the small yellow follicles in the CAP PF ducks was significantly higher than that of the other 2 groups ($P < 0.01$). The relative weight of the small yellow follicles in the CAP free-fed ducks was not significantly different from the control group ($P > 0.05$). Ducks that were AF fed with CAP-supplemented diets had a higher number of dominant follicles than the control ($P = 0.06$). The mRNA expression of *TRPV4*, *ATP2A2*, *ITPR1*, and *CaM* in the CAP AF fed ducks were significantly higher than the other 2 groups ($P < 0.05$, Table 5). The ovarian mRNA expression of *CDK1* in CAP AF fed ducks was significantly higher than that of the other 2 groups ($P = 0.01$, Table 6). The ovarian mRNA expression of cyclin B2 (***CCNB2***) in the CAP PF group was significantly higher than that of the other 2 groups ($P < 0.01$), but AF fed CAP had no effects on the mRNA expression of *CCNB2*.

As shown in Table 7, ducks fed CAP-supplemented diets had higher plasma glutathione peroxidase activity ($P < 0.01$) than the control but had lower MDA content in the ovaries tissue ($P < 0.01$). Capsaicin supplementation to diets, when diets were ad libitum or pair fed, had no effects on either the plasma estradiol or progesterone concentrations in laying ducks (Table 8). Similarly, CAP had no effects on ovarian mRNA expression of *FOXL2*, *STAR* or *CYP19A1*, which are related to estrogen synthesis (Table 9).

Table 3. Effects of capsaicin on the productive performance of 58- to 64-wk-old laying ducks.[1]

Variables	CON	150 mg capsaicin/kg diet		SEM	*P*-value
		PF	AF		
Daily feed intake, g/d	154[b]	154[b]	166[a]	0.43	<0.01
Egg production, %	77.3	78.7	82.7	1.45	0.06
Egg weight, g/egg	68.0	67.3	68.7	0.38	0.08
Egg mass, g/d	52.8	53.9	56.8	1.27	0.11
FCR, g feed/g egg	2.91	2.86	2.76	0.06	0.30

[a,b]Means within a row with different superscript letters differ significantly ($P < 0.05$).

Abbreviations: AF, *ad libitum* fed; CON, basal control groups without supplementation of capsaicin; FCR, feed conversion ratio; PF, pair fed to control treatment.

[1]Data are means for n = 6 replicates (12 individually caged laying ducks/replicate).

DISCUSSION

In this study, CAP supplementation increased feed intake of laying ducks when feed was provided AF. Egg production performance tended to be improved by CAP under a condition that ducks has free access to feed. This suggests that the positive effect of CAP on egg production performance was probably, at least in part, owing to the improved feed intake. In accordance with the improved egg production performance, large yellow follicle number tended to be increased by CAP under the condition of free access to feed. Similar to our results, it was found that quail that received diets with 1.2 g red pepper oil consumed more feed than the others (Reda et al., 2019). It was reported that the inclusion of hot red pepper in the broiler chickens diets increased BW, feed intake, and improved FCR (Al-Kassie et al., 2011). It is, therefore, indicated that CAP can stimulate the appetite of laying ducks and increased the feed intake. Ducks that were AF fed with CAP-supplemented diets tended to have higher egg production and egg weight.

The transient receptor potential (**TRP**) protein is a nonspecific phosphoinositide-mediated Ca^{2+}-permeable channels (Minke, 2006). The transient receptor potential vanilloid subfamily (**TRPV**) contains 6 proteins (TRPV1–V6) in mammals, and they exhibit functional similarities and Ca^{2+}-selectivity (Wu et al., 2010). They mediate behavioral responses to exogenous chemical, mechanical, and temperature stimuli. Capsaicin was demonstrated to be a highly selective agonist for TRPV1 (Caterina et al., 1997; Yang et al., 2010), and the activation of TRPV1 by CAP is dependent on Ca^{2+}/calmodulin (Rosenbaum et al., 2004). In this study, interestingly, the mRNA of *TRPV4* rather than *TRPV1* was detected in the ovary of ducks and the mRNA expression of *TRPV4* was increased by AF fed CAP, indicating the ovarian TRPV4 was activated by dietary CAP supplementation. The genes related to Ca-mediated signaling pathway, downstream of TRPV4, were also assayed in ovary. Similarly, mRNA

Table 4. Effects of capsaicin on the ovarian indices of 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
Large yellow follicles number, diameter >8 mm	3.50	4.90	5.00	0.42	0.06
Small yellow follicles number, 3 mm < diameter <8 mm	21.5	18.2	17.9	1.71	0.30
Number of atrestic follicles	2.67	2.60	1.30	0.53	0.17
Ovarian weight, g/kg live BW	24.5	24.8	33.4	3.52	0.18
Large yellow follicle weight/ovarian weight, %	75.8^{b}	86.2^{a}	88.3^{a}	2.53	0.01
Small yellow follicle weight/ovarian weight, %	5.67^{b}	14.1^{a}	3.67^{b}	1.80	<0.01

^{a,b}Means within a row with different superscript letters differ significantly ($P < 0.05$).

Abbreviations: AF, *ad libitum* fed; CON, basal control groups without supplementation of capsaicin; PF, pair fed to control treatment.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

expression of *ATP2A2*, *ITPR1*, and *CaM* was increased by AF fed CAP but not by PF CAP. Because the Ca-mediated signaling pathways are important in affecting follicle selection and maturation in laying birds (Chen et al., 2020), we speculate that CAP may play a role in promoting follicle growth, at least via activating TRPV4-mediated Ca signaling pathway.

Cyclin-dependent kinase 1 (**CDK1**) is a cyclin-dependent kinase and a major cell cycle regulator, from the cyclin-dependent kinase family. The cyclin-dependent kinase family (including CDK1 through CDK20) is a serine–threonine kinase that regulates the G1/S and G2/M cell cycles by forming active cyclin-dependent kinase–cyclin complexes. Previous studies have shown that increased CDK1 protein kinase activity leads to nuclear changes associated with oocyte maturation and mitosis (Lohka, 1998). Cyclin-dependent kinase 1 can bind cyclin B1 (**CCNB1**) and cyclin B2 (**CCNB2**) at different stages (Maleszewska et al., 2016). Cyclin B2 is a cycle-related protein; high expression of CCNB2 and other genes indicates the beginning of mitosis in the cell cycle. In this study, when laying ducks were AF fed CAP, the ovarian mRNA expressions of *CDK1* and *CCNB2* in the ovaries were significantly upregulated, suggesting that CAP could increase the proliferation of follicular cells (granulosa cell, perhaps) and promote the growth and maturation of follicles.

In this study, CAP significantly increased the plasma glutathione peroxidase activity and reduced the plasma MDA content in laying duck, which indicates that CAP can improve the redox status in laying duck, and it works better under AF feeding conditions. The present data are in accordance with those of Abdelnour et al. (2018), who reported that dietary supplementation of red pepper oil caused a decrease in MDA levels and an increase in serum antioxidant enzyme activities. Similar to our results, it was reported that dietary supplementation of red pepper oil (0.8 g/kg) could enhance the performance and antioxidant indices, improve lipid profile, and decrease intestinal pathogens, thus improving the health status of growing Japanese quail (Reda et al., 2019). Capsaicin has been demonstrated previously in vivo and in vitro to exert positive effects on animal antioxidants. For instance, CAP protects red blood cells from tert-butylhydroperoxide (**T-BHP**)–induced oxidative stress in culture medium (Luqman and Rizvi, 2006), reduces MDA in guinea pigs (Yang et al., 2018), and protects venous endothelial cells from oxidative stress (Chen et al., 2015a). Dietary CAP supplementation in rats can attenuate carbon tetrachloride (**CCL4**)-induced liver injury by enhancing the activities

Table 5. Effect of capsaicin on ovarian mRNA expression abundance of genes related to TRPV activation in the ovary of 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
TRPV4	1.00^{b}	1.26^{b}	2.50^{a}	0.363	0.03
CALB1	1.00	1.70	1.22	0.230	0.08
ATP2A2	1.00^{b}	1.02^{b}	2.73^{a}	0.196	<0.01
Oral1	1.00	1.43	1.29	0.193	0.31
ITPR1	1.00^{b}	1.03^{b}	1.83^{a}	0.183	<0.01
CaM	1.00^{b}	1.06^{b}	1.939^{a}	0.192	<0.01

^{a,b}means within a row with different superscript letters differ significantly ($P < 0.05$).

Abbreviations: AF, *ad libitum* fed; *ATP2A2*, *ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2*; *CALB1*, *Calbindin 1*; *CAM*, *Calmodulin*; CON, basal control groups without supplementation of capsaicin; *ITPR1*, *Inositol 1,4,5-Trisphosphate Receptor Type 1*; PF, pair fed to control treatment; *TRPV4*, *the transient receptor potential vanilloid subfamily* 4.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

Table 6. Effects of capsaicin on the ovarian mRNA expression abundance of genes related to follicle development in 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
CDK1	0.99^{b}	1.06^{b}	1.37^{a}	0.08	0.01
CCNB2	1.11^{b}	1.74^{a}	1.29^{b}	0.13	<0.01
Bcl2	0.99	1.15	0.98	0.22	0.83
Caspase3	1.34	1.29	1.01	0.20	0.48
FAS	0.90	1.11	0.99	0.09	0.27

^{a,b}Means within a row with different superscript letters differ significantly ($P < 0.05$).

Abbreviations: AF, ad libitum fed; *Bcl2*, *BCL2 apoptosis regulator*; *CCNB2*, *Cyclin B2*; *CDK1*, *cyclin dependent kinase 1*; CON, basal control groups without supplementation of capsaicin; *FAS*, *Fas cell surface death receptor*; PF, pair fed to control treatment.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

Table 7. Effects of capsaicin on plasma and ovarian antioxidant indexes of 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
Plasma					
T-AOC, U/mL	7.55	7.61	9.26	0.81	0.26
T-SOD, U/mL	5.36	5.26	5.24	0.11	0.70
Gpx, U/mL	404[c]	430[b]	451[a]	5.89	<0.01
MDA, nmol/mL	3.34	3.20	2.67	0.26	0.19
Ovary					
T-AOC, U/mg protein	7.37	8.38	8.20	0.87	0.69
T-SOD, U/mg protein	160	166	164	10.4	0.93
Gpx, U/mg protein	316	314	306	11.0	0.80
MDA, nmol/mg protein	8.49[a]	6.06[b]	2.65[c]	0.74	<0.01

[a,b]Means within a row with different superscript letters differ significantly ($P < 0.05$).

Abbreviations: AF, ad libitum fed; CON, basal control groups without supplementation of capsaicin; Gpx, glutathione peroxidase; MDA, malondialdehyde; PF, pair fed to control treatment; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

of superoxide dismutase, catalase, and glutathione-S-transferase (Hassan et al., 2012). Capsaicin is observed to inhibit copper ion–induced lipid peroxidation of human low-density lipoprotein (Naidu and Thippeswamy, 2002). This suggest that CAP is an effective antioxidant and offer protection against oxidation of human low-density lipoprotein. Wistar rats administered CAP (*i.p.* 3 mg/kg BW) for 3 consecutive d showed a reduction of oxidative stress measured as MDA in the liver, lung, kidney, and muscle (Lee et al., 2003). The beneficial influence of CAP on the antioxidant status of red blood cells and the liver in induced hypercholesterolemic rats is also evidenced (Kempaiah and Srinivasan, 2004). Capsaicin was also found to scavenge 1,1′-diphenyl-2-picrylhydrazyl radicals both at/near the membrane surface and in the interior of the membrane. Vanillin and 8-methyl-6-noneamide were major reaction products of CAP with 1,1′-diphenyl-2-picrylhydrazyl radicals, thus suggesting that the radical scavenging site of CAP is the C7-benzyl carbon. Phenolic compounds of various spices, including CAP modulate 5-lipoxygenase in human polymorphonuclear leukocytes (**PMNL**) cells, the key enzyme involved in the biosynthesis of leukotrienes (Prasad et al., 2004). The antioxidant function of CAP has been well summarized in the review of Srinivasan (2016).

Table 8. Effects of capsaicin on the plasma concentration of steroid hormone in 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
E2 (pg/mL)	340	508	512	76.6	0.23
Progesterone (ng/mL)	0.15	0.15	0.17	0.02	0.59

Abbreviations: AF, ad libitum fed; CON, basal control groups without supplementation of capsaicin; E_2, estradiol-17 beta; PF, pair fed to control treatment.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

CONCLUSION

The dietary supplementation with CAP under condition of ad bilitum feeding increased egg production due to enhanced the follicular growth and maturation, the process of which is related to activation of TRPV4 and Ca signaling pathway in ovary, as well as improvement in antioxidant capacity.

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Table 9. Effects of capsaicin on the ovarian mRNA expression abundance of genes involved in estrogen synthesis in 64-wk-old laying ducks.[1]

Item	CON	150 mg/kg capsaicin		SEM	*P*-value
		PF	AF		
FOXL2	0.90	1.15	1.19	0.22	0.71
STAR	0.73	0.84	1.06	0.15	0.69
CYP19A1	0.79	0.97	1.16	0.14	0.27

Abbreviations: AF, ad libitum fed; CON, basal control groups without supplementation of capsaicin; *CYP19A1*, *Cytochrome P450 family 19 subfamily A member 1*; *FOXL2*, *Forkhead Box L2*; PF, pair fed to control treatment; *STAR*, *steroidogenic acute regulatory protein*.

[1]Data are means for n = 6 replicates (2 ducks/replicate).

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DISCLOSURES

The authors declare no conflicts of interest.

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