Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2019 / v.21 / n.4 / 001-010

http://dx.doi.org/10.1590/1806-9061-2018-0892

Original Article

Effects of Vitamin E Supplementation on Serum Hormones and Gene Expression of Anti-season Breeding Xingguo Grey Geese (Anser cygnoides)

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■Keywords

Vitamin E, Reproductive hormones, Ovary, Follicle, Dicer.

Submitted: 21/February/2019
Approved: 18/August/2019

ABSTRACT

The objective of this study was to analyse the impact of dietary vitamin E supplementation on laying performance, serum reproductive hormones concentration and gene expression in ovary and follicles of anti-season breeding goose. A total of 210 anti-season breeding geese were divided into seven treatments with six replications. Each group was supplied with diets containing different vitamin E (DL-α-tocopherol acetate) contents (0, 10, 20, 40, 80, 160, 320mg/kg). We observed that the egg production and laying rate improved significantly at doses of 10 and 80 mg/kg, while the highest egg weight appeared in the 320 mg/kg group. Meantime, 80 mg/kg of vitamin E supplementation significantly improved the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) in serum (p<0.05). Dietary vitamin E supplementation significantly enhanced mRNA expression of *FSHR*, *LHR* and *ESR1* at a dose of 80mg/kg, while *PRLR* increased at doses of 10 and 20mg/kg (p<0.05). It was found that the mRNA expression of *Dicer* increased at doses of 40 and 80 mg/kg of vitamin E supplementation in the ovary, SWF, LWF and SYF, respectively. Thence, Dietary vitamin E supplementation could improve egg laying performance, plasma reproductive hormones and the mRNA expression of reproductive hormone receptor genes in ovary, as well as the mRNA expression of *Dicer* in ovary, SWF, LWF and SYF. It was supposed that 80 mg/kg of vitamin E supplementation in dietary was appropriate to improve the fertility of anti-season breeding Xingguo grey goose.

INTRODUCTION

The egg production of poultry is an essential economic problem in husbandry. Caused by the seasonal reproduction, the development of goose industry is sluggish compared with chicken in nowadays breeding industry. With respect to this issue, a valid method called "short illumination control technology and forced moulting method was applied to accomplish anti-season reproduction of goose. Xingguo Grey Goose is a popular indigenous breed distributed in Jiangxi Province. With a strong seasonal reproduction characteristic, the annual egg production of Xingguo Grey Goose is approximately 30-40 eggs per goose. It has been reported that Xie and colleagues used the short illumination control technology and forced moulting method to accomplish the anti-season breeding of Xingguo Grey Goose (Xie *et al.*, 2011). In consideration of the broody stages of Xingguo Grey Goose from May to August, herein, we adopted this method to regulate the reproductive cycle of Xingguo Grey Goose for anti-season breeding. Ceasing egg-laying is usually observed in geese during the non-breeding period because of the decrease of follicles. After the non-breeding period, the secretion of several reproductive hormones, such as FSH and LH are increased (Xie *et al.*, 2011), which promotes follicles

to increase and develop rapidly in the ovary. Hence, we detected the concentration of reproductive hormones after vitamin E treatment in this study.

Vitamin E consists of four tocopherols and four tocotrienols, of which α-tocopherol is the main biologically active form. Since it was discovered in the 1920s, vitamin E has demonstrated to own numerous functions, such as anti-oxidation, cellular immune regulation and lipids transportation. In humans, adding vitamin E (α-tocopherol) to endothelial cells and fibroblasts cells in vitro could reduce the numbers of senescent cells and protect against $H_{(2)}O_{(2)}$-induced DNA damage and telomere shortening (Makpol *et al.*, 2010). In pigs, vitamin E integrated with other elements could reduce percent peroxide hemolysis, serum cholesterol and fatty acid levels (Young *et al.*, 1976). In ruminants, the supplementation of vitamin E was thought to affect the numbers of some immune cell types in the peripheral blood of suckling Japanese black calves (Otomaru *et al.*, 2015). It was also suggested that the saturated fatty acid decreased and monounsaturated fatty acid increased when Aohan fine-wool sheep supplied with 200 IU vitamin E (Liu *et al.*, 2013). As for poultry, the available evidence seems to point to the essential role of vitamin E in meat quality, growth performance, lipids metabolism, immunity and fertility. High levels of vitamin E (150mg/kg) in the diets could improve the antioxidant function and lipids metabolism of Guangxi Sanhuang chicken (Liu *et al.*, 2017). Biswas's findings claimed that moderate supplementation of dietary vitamin E (100mg/kg) may improve physical and biochemical characteristics of semen in Indian reared KN cock (Biswas *et al.*, 2009). However, the impact of vitamin E on goose reproduction is relatively less reported.

Dicer, an RNase III endonuclease, is critical for small noncoding RNAs (micro RNAs and small interfering RNAs) synthesis, as well as regulating mRNA expression in transcriptional and post-transcriptional modifications via binding to target mRNA and leading to subsequent mRNA degradation (Song & Rossi, 2017). Currently, *Dicer* was also reported to involve in follicular development, ovulation, luteinization and reproductive hormone synthesis (Kurzynska-Kokorniak *et al.*, 2015). Otherwise, *Dicer* could also affect the development and function of corpus luteum via corpus luteum angiogenesis (Otsuka *et al.*, 2008). Although *Dicer* may play a critical role in fertility, especially in gonadotropin homeostasis, fewer reports related to the effect of vitamin E supplementation on the expression of *Dicer* in ovary and follicles was found.

Generally, we employed the anti-season breeding Xingguo Grey Goose to investigate the effects of vitamin E supplementation on plasma reproductive hormones concentration and the expression of reproductive hormones genes and *Dicer* in ovary and follicle. Further to reveal the effect of vitamin E supplementation on the fertility of goose at physiological level.

MATERIALS AND METHODS

All experimental protocols involving animals in the present study were approved by the Committee on the Care and Use of Laboratory Animals of the State-Level Animal Experimental Teaching Demonstration Centre of Sichuan Agricultural University and Jiangxi Academy of Agriculture Sciences. All methods involved in this study were conducted according to the Regulations of the Administration of Affairs Concerning Experimental Animals.

Animal treatment

All experiment animals were raised in Xingguo Grey Goose Research Base (annual temperature average is 18.9 °C, 115°35'E, 26°33'N). 210 healthy laying geese aged 45 weeks (in the middle of egg production period) were selected and treated with forced moulting method and short illumination(13L: 11D) established by Xie and colleagues (Xie, Liu, Yan-Ping, Zhou, Xie, Wei, Kang, Hua-Yuan, Huang, Zeng, 2011) to regulate the reproductive cycle of Xingguo grey goose. After a recovery period of about four weeks, 210 geese were randomly divided into seven groups (6 replicates, 5 observations). All geese were supplied with a commercial ration (Table 1) containing increasing levels of vitamin E supplementation (0, 10, 20, 40, 80, 160,

Table 1 – Composition and nutrient levels of experimental diets (air-dry basis).

Ingredients	Percentage (%)
Corn	40
Paddy	40
Soybean meal	16.04
stone dust	1.44
Calcium phosphate dibasic	1.52
Premix [1)]	1
Constituents	
ME(MJ/Kg)	11.39
Crude protein(CP)	14.11
Ca	0.94
P available	0.62

[1)] Contained the following per kg of premix: Cu (as copper sulfate) 10 mg, Fe (as ferrous sulfate) 90 mg, Se (as sodium selenite) 0.2 mg, Zn (as zinc sulfate) 80 mg, VA 9 000 IU, VD_3 2 000 IU, VE 20 IU, VK 2.50 mg, $VB_1$2.50 mg, VB_2 4.50 mg, VB_6 4 mg, pantothenic acid 10 mg, choline 800 mg, nicotinic acid 50 mg and folic acid 1 mg.

320 mg/kg). Seven treatments were kept in a light-shelter room and exposed to an artificial short lighting program (11L:13D) with *ad libitum* access to feed and water. This treatment lasted for 12 weeks until all geese went into egg-laying period again. To calculate the laying rate of each group, the egg production and egg weight were recorded for three weeks in each group.

Blood sample collection and serum hormone concentration analysis

We collected six laying geese from each treatment. Each bird was sampled three times, with a 48 h sampling interval, which is considered as the theoretical oviposition cycle. All the blood samples were collected from the wing vein using heparinized syringes. To separate the serum, we centrifuged the blood samples at 3000xg for 10 min at 4°C and stored at –20°C until analysis. The ELISA kits (Kenuodi, Fujian, China) with the intra-assay and inter-assay were used to evaluate the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and estradiol (E2) in serum. The coefficient of variation was less than 15% precision. A total of 42 samples were measured in triplicates for each sample. The enzyme-labelled instrument (Thermo Fisher, Varioskan™ LUX, Germany) was used to measure absorbance at 450nm.

Ovarian tissues collection, RNA extraction, cDNA synthesis, and real-time PCR analysis

A total of 42 fowls (6 fowls for each treatment) were selected randomly and slaughtered for ovary and follicle tissues collection. The ovarian tissues were separated, small white follicles (SWF,1 mm≤d≤3mm), large white follicles (LWF,6 mm≤d≤10 mm) and small yellow follicles (SYF,10 mm≤d≤20 mm), were frozen in liquid nitrogen immediately, and stored at -80°C until analysis. Total RNAs were extracted using the Trizol RNA extraction reagent (Invitrogen Corp., CA, USA) and reverse transcribed to cDNA using PrimeScript™ RT reagent Kit (Perfect Real-Time, Takara, Dalian, China) in accordance with the manufacturer's instruction. Real-time quantitative PCR analysis of follicle-stimulating hormone receptor (*FSHR*), luteinizing hormone receptor (*LHR*), prolactin receptor (*PRLR*), estradiol receptor 1 (*ESR1*) and *Dicer* mRNA were performed with β-actin as the internal control standard. The primers of target genes were listed in Table 2. Real-time quantitative PCR was conducted on ABI 7500 Fluorescent Quantitative PCR system (Applied Biosystems, Bedford, MA, USA) using a 20μL reaction volume with SYBR Green I Master Mix (Toyobo, Osaka, Japan). The real-time quantitative PCR conditions were: 95°C pre-denaturation for 5 minutes, 40 cycles of 95°C for 15 s, 60°C for 35 s, and melting curves were obtained at 60°C for 60 s to 95°C for 15 s. All assays were performed in triplicate. The $2^{-\Delta\Delta Ct}$ ($\Delta Ct = Ct_{gene} - Ct_{\beta\text{-}actin}$, Ct: cycle threshold values) was represented as the relative quantification of gene amplification.

Statistical analysis

All statistics were disposed in EXCEL 2010 and then analysed by one-way ANOVA of SAS Software Version 9.2 (SAS Institute Inc. Cary, NC. USA). Variance analysis was performed to evaluate the differences, and Duncan post hoc test was employed to determine the effects of treatments at 1% or 5% level of significance ($p<0.01$, or $p<0.05$). All results were presented as mean ± SD (standard deviation of the mean).

RESULTS

Egg-laying performance

The effect of different levels of dietary vitamin E supplementation on egg laying performance was

Table 2 – Primers used in real-time quantitative PCR of genes in geese samples[1)]

Gene name	Accession number	Prime sequences (5′→3′)	Annealing temperature (°C)	PCR product (bp)
Dicer	XM_013181712.1	Forward primer: TGCCCACAGAATACCGTTCC	60.04	193
		Reverse primer: GGTTCAGTTTCGGTTTCGCC	60.04	
ESR1	XM_013178336.1	Forward primer: TCCAGTGTACGGCTCTACCA	59.96	120
		Reverse primer: TGCAAGAACACCACAGGGTT	60.03	
LHR	XM_013192443.1	Forward primer: TTACAAGCTCACGGTTCCCC	59.96	255
		Reverse primer: TGCATGGCGTAGGTGATTGT	60.04	
FSHR	XM_013192472.1	Forward primer: ATGTTTGCTTTCACGGTGGC	59.97	275
		Reverse primer: GCCATGCAGAGGAAGTCTGT	60.04	
PRLR	XM_013181699.1	Forward primer: AAGGAGCCGGGAAACTCAG	59.96	198
		Reverse primer: CCACCAAAACTGGGGCAATG	59.96	
β-actin	NW_013185670.1	Forward primer: AGAGGTGGGAACCACACTTG	59.53	162
		Reverse primer: TCCCCTTGTCAAAGCACTCC	59.89	

[1)] ESR1: estradiol receptor 1; LHR: luteinizing hormone receptor; FSHR: follicle-stimulating hormone receptor; PRLR: prolactin receptor.

presented in Table 3. Different dietary levels of vitamin E supplementation had significant effect on the egg production, laying rate and average egg weight of Xingguo geese. The egg production and laying rate at doses of 10 and 80 mg/kg vitamin E supplementation were extremely significantly higher than the control group (p<0.01) but decreased at doses of 20 and 160 mg/kg vitamin E addition (p<0.05). The highest average egg weight was observed in 320 mg/kg group, which increased significantly compared with the control one (p<0.05).

Table 3 – Egg-laying performance of the geese[1)]

VE (mg/kg)	Egg production	Laying rate	Average egg weight
0	20.67±3.56	0.20±0.034	171.02±3.09
10	26.17±2.48**	0.25±0.023**	170.81±2.02
20	17.33±1.21*	0.17±0.012*	169.38±4.23
40	22.00±2.19	0.21±0.021	168.91±2.43
80	27.33±1.63**	0.26±0.016**	165.73±1.79*
160	17.83±0.41*	0.17±0.003*	168.71±5.65
320	18.83±2.56	0.18±0.024	176.16±3.51*

[1)]Egg-laying performance was presented as mean ± SD (standard deviation of the mean)(n=6). "*" or "**" on the row denotes significant difference (p<0.05) or extremely significant difference (p<0.01).

Concentration of reproductive hormones in serum

The data presented in Table 4 showed that different levels of vitamin E supplementation had effects on serum reproductive hormones in anti-season breeding geese. The FSH concentration was extremely significantly higher at doses of 40, 80 and 160 mg/kg than in the control group (p<0.05) and showed a peak at the dose of 80 mg/kg. The results (Table 4, Figure 1A) suggest that FSH concentration at the dose of 80 mg/kg was approximately 15 times higher than in the control from 0.38±0.29 to 5.87±1.49 mIU/ml. Similar to the trend of FSH, the concentration of E2 also increased at doses of 20, 40, 80 and 160 mg/kg compared with the control group. The concentration of LH only increased significantly at the dose of 80 mg/kg (p<0.05). Whereas PRL concentration (Table 4, Figure 1B) increased from 0 mg/kg (5.72±0.41) to 20mg/kg (15.41±3.19), and a peak was showed at 20 mg/kg. Then PRL concentration decreased from 20 mg/kg to 40 mg/kg, but when the dose above 40 mg/kg, the concentration of PRL increased again.

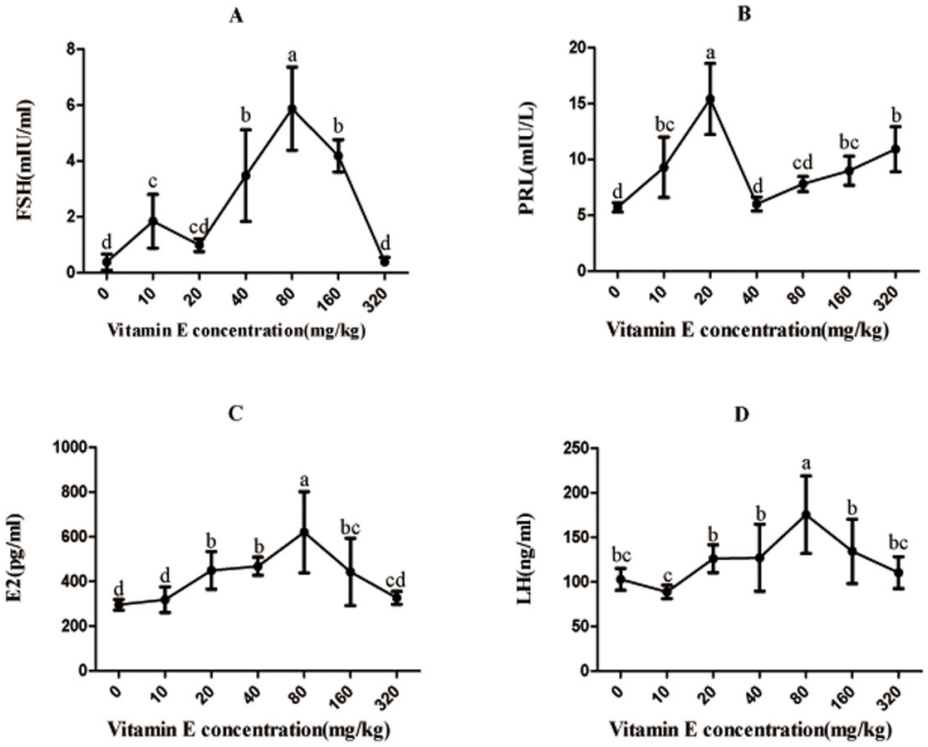

Figure 1 – Serum concentrations of FSH, PRL, E2, LH during the oviposition or ovulation cycle. Vertical bars represent standard deviation of the mean. Means not marked by a common letter are significantly different (p< 0.05).

Table 4 – Serum hormone concentration of the geese[1)]

variable	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
FSH(mIU/ml)	0.38±0.29	1.84±0.96*	0.99±0.23	3.47±1.640**	5.87±1.49**	4.19±0.58**	0.39±0.16
PRL(mIU/L)	5.72±0.41	9.29±2.71B**	15.41±3.19**	6.01±0.62	7.80±0.68	8.99±1.30**	10.92±2.03**
E2(pg/ml)	295.83±23.77	318.75±57.37	449.77±84.39*	468.29±40.51*	620.37±181.35**	443.06±151.04*	326.85±29.19
LH(g/ml)	102.99±12.27	88.85±7.41	126.16±15.55	127.29±37.52	175.48±43.59**	134.41±36.21	110.53±17.98

[1)]Serum hormone concentration was presented as mean ± SD (standard deviation of the mean)(n=6). FSH, follicle-stimulating hormone. PRL, prolactin. E2, estrogen. LH, luteinizing hormone. "*" or "**"on the row denotes significant difference (p<0.05) or extremely significant difference (p<0.01).

Reproductive hormone gene expression in the ovary

The relative mRNA expression of *FSHR*, *PRLR*, *ESR1* and *LHR* in the ovary affected by dietary vitamin E supplementation is shown in Figure 2. Compared with the control one, the relative mRNA expression of *ESR1*, *FSHR* and *LHR* significantly increased by dietary vitamin E supplementation at the dose of 40 or 80 mg/kg (p<0.05). The result showed that there was no significant difference of *ESR1*and *LHR* relative mRNA expression was observed between 40 and 80 mg/kg. Conversely, *PRLR* relative mRNA expression significantly increased at doses of 10 and 20 mg/kg (p<0.05). Dietary vitamin E supplementation at 40 and 80 mg/kg had no significant impact on *PRLR* mRNA expression in the ovary, which was in contrast to the results of *FSHR* and *ESR1*. Among the four genes, *FSHR* showed higher relative mRNA expression in ovary compared with the others, and the relative mRNA expression of *PRLR* in the ovary was the lowest.

Dicer expression in ovary, SWF, LWF, SYF

As a transcriptome regulator, *Dicer* was supposed to participate in follicular growth and development. The effects of dietary vitamin E supplementation on *Dicer* relative mRNA expression in the ovary, small white follicle (SWF), large white follicle (LWF) and small yellow follicle (SYF) are presented in Figure 3. In the ovary, compared with the control group, the relative mRNA expression of *Dicer* significantly increased at the dose of 40 mg/kg and decreased at 320 mg/kg (p<0.05). In SWF and LWF, differing from other treatments, the relative mRNA expression of *Dicer* significantly increased at 80 mg/kg in contrast with the control group (p<0.05). In SYF, the relative mRNA expression

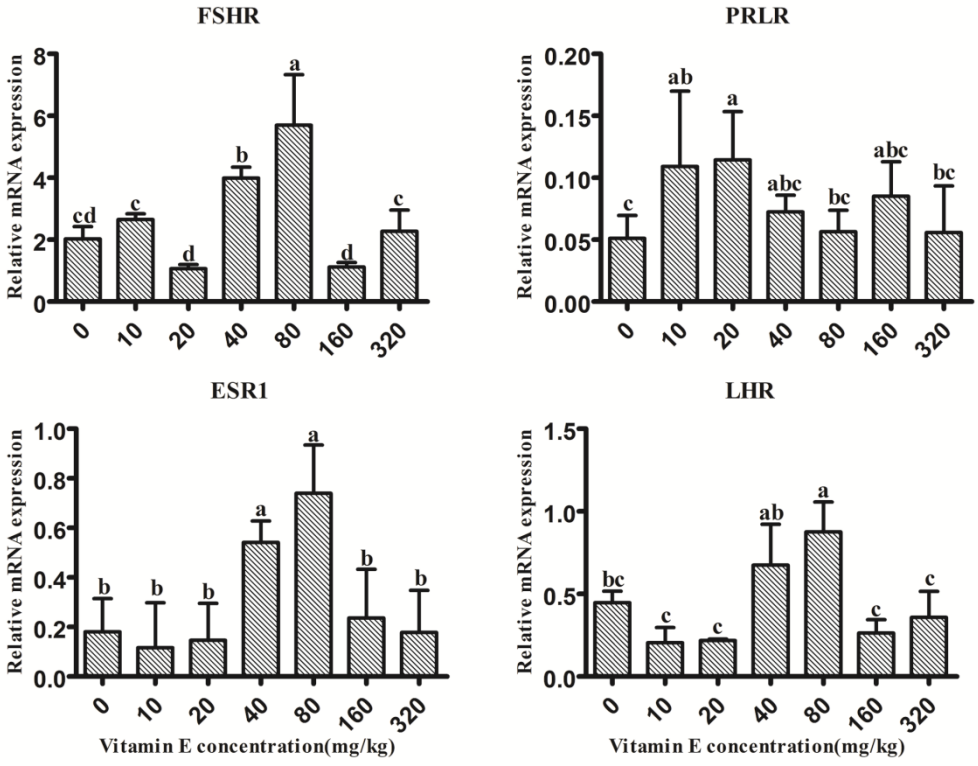

Figure 2 – The mRNA expression level of FSHR, PRLR, ESR1, LHR relative to β-actin in ovary of anti-season breeding Xingguo grey goose. Vertical bars represent standard deviation of the mean. Means not marked by a common letter are significantly different (p< 0.05).

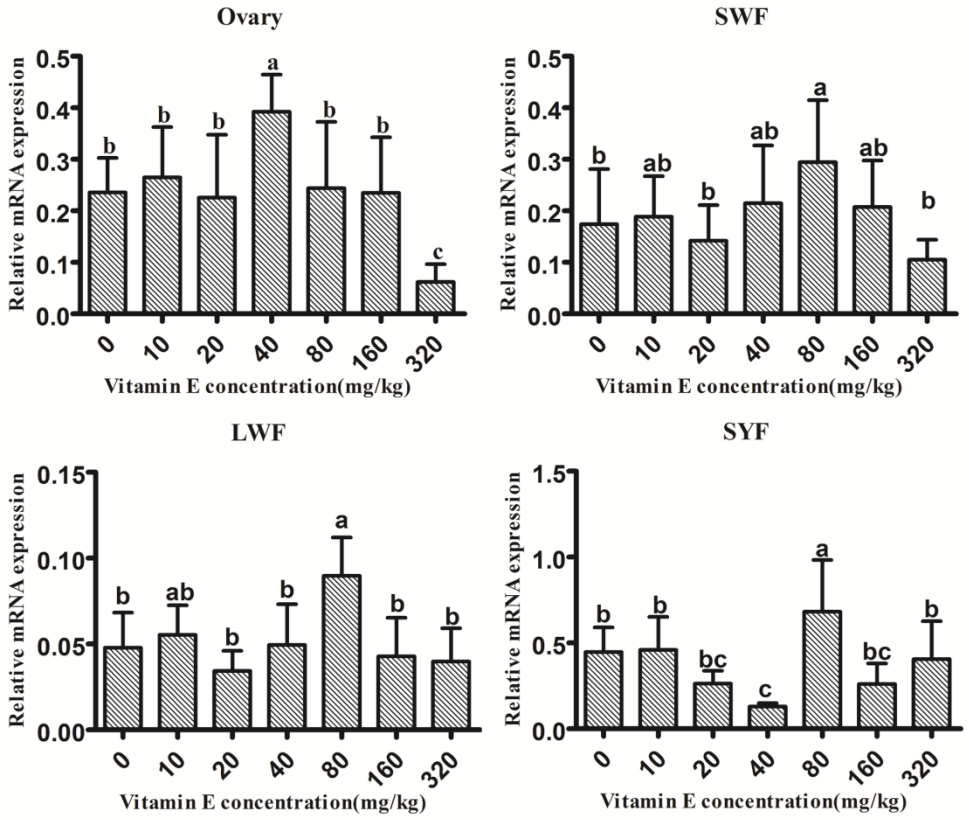

Figure 3 – The mRNA expression level of Dicer relative to β-actin in ovary, SWF, LWF, SYF of anti-season breeding Xingguo grey goose. Vertical bars represent standard deviation of the mean. Means not marked by a common letter are significantly different (p<0.05).

of *Dicer* presented a tendency to decrease when the dose of vitamin E supplementation increased from 0 mg/kg to 40 mg/kg. The dose of 40 mg/kg vitamin E supplementation significantly suppressed the relative mRNA expression of *Dicer* compared with the control group (p<0.05). Otherwise, among the four tissues, the relative mRNA expression of *Dicer* was observed to express less in LWF and higher in SYF without vitamin E supplementation.

DISCUSSION

Vitamin E, the most abundant radical-scavenging antioxidant in vivo, is thought to affect the reproduction in humans and animals. It was described as that 30 and 60 mg/kg doses of dietary vitamin E had a significant impact on egg production rates and embryonic viability (Abedi *et al.*, 2017). Biswas *et al.,* reported that hens supplemented with 150 IU/kg VE significantly improved egg production and fertility (Biswas *et al.*, 2010). Nevertheless, according to Hooda *et al.*, 75, 150, 225 or 300 IU/kg vitamin E addition had no effect on the fertility and hatchability of Japanese quails (Hooda *et al.*, 2007).Currently, supplementation of 10 and 80 mg/kg of vitamin E had an exceeding significant impact on the egg production and laying rate. However, when the geese were supplied with 20 and 160 mg/kg of vitamin E, the egg production and laying rate were lower than the zero control. This result was not completely consistent with the variation of serum FSH and LH content (Figure 1), which was said to regulate the follicle development and ovulation. This can be interpreted as that the formation of eggs was a complex biological process, which was not only regulated by gonadal hormone levels. Although the egg production and laying rate increased at a dose of 80 mg/kg of vitamin E addition, the egg weight was the lowest. While the egg weight in 320 mg/kg group was terrific in spite of the egg production being lower than that in the 80 mg/kg group, confirming the results of previous studies that egg production was found to be negatively correlated with egg weight (Singh *et al.*, 2000, Veeramani *et al.*, 2012, Tongsiri *et al.*, 2015).

Vitamin E influenced chicken's sexual function by regulating the secretion of gonadotropins from the anterior pituitary. The researches have shown that supplementation of dietary vitamins E enhanced the size and area of FSH and LH gonadotropic cells and modulated the function state of gonadotropic cells (Mumford *et al.*, 2015). Herein, the gonadotropins

FSH concentration in serum were improved by dietary vitamin E complement at doses of 20-160 mg/kg in anti-season breeding Xingguo grey geese and LH was at the dose of 80 mg/kg, indicating that apposite vitamin E addition may impact the synthesis and release of gonadotropin in anterior pituitary. FSH mainly regulated follicular development and selection. The literature on Hardy *et al.* proved that FSH was essential for pre-antral follicle growth and function in mice (Hardy *et al.*, 2017). In poultry, FSH treatment increased the embryonic chicken ovarian germ cell number, granulosa cells number and reduced cell apoptosis in different stage of follicles(Lin *et al.*, 2011). In this study, the increase of FSH and LH in geese may induce follicular development and ovulation which may result in the increase of egg production of goose.

In addition, estrogen was also essential to stimulate granulosa cell proliferation and facilitate the function of FSH and LH in follicles (Richards, 1980). Previous researches showed that α-tocopherol was positively associated with E2 and free E2 in healthy women (Mumford, Browne, Schliep, Schmelzer, Plowden, Michels, Sjaarda, Zarek, Perkins, Messer, 2015). In our findings, the concentration of E2 was increased at doses of 20-160 mg/kg of vitamin E supplementation. In accordance with the function of vitamin E in organisms, it was conjectured that vitamin E may affect the concentration of E2 in three aspects. Firstly, vitamin E protected the structural integrity of theca cells, a kind of cell mainly for the synthesis and secretion of estradiol, through scavenging the free radicals to oxidize polyunsaturated fatty acids and lipoproteins on the membrane. For the second aspect, as a main ingredient for the synthesis of estradiol, the transport of cholesterol is consistent with vitamin E transportation in intestinal epithelial cells, which improves the synthesis of estradiol. Furthermore, granular cell, an essential role in follicle, functioned as regulating the development of follicle and promoting the synthesis of steroid hormones (Onagbesan *et al.*, 2009). As previous findings reported that the proliferation of granular and theca cells were regulated by FSH and LH (Drummond, 2006). This means that serum E2 concentration may indirectly regulate by the concentration of FSH and LH as well.

PRL, secreting from lactotroph cell of the anterior pituitary, was confirmed to induce broodiness in birds (El Halawani *et al.*, 1980). As described previously, PRL expression pattern in contrast with E2 increased in broodiness and decreased in the breeding period (Chen *et al.*, 2005).The synthesis and secretion of prolactin were regulated by Pou1f1 transcription factor, dopamine, estradiol and other factors (Scully *et al.*, 1997, Denef, 2008, Numan & Woodside, 2010). High concentration PRL secretion in broodiness suppressed the release of LH, E2 and progesterone in ovary and reduced the number of normal follicles (Sharp *et al.*, 1988), which may suppress a series of reproductive activities. In this study, the pattern of serum PRL concentration was distinctive, which increased at low vitamin E supplementation (10 and 20 mg/kg) and reduced at intermediate and high level (>20 mg/kg). This result may be explained as the changes of E2 concentration caused by the influence of vitamin E supplementation affected the expression of PRL gene (Lieberman *et al.*, 1981). Otherwise, the acute changes in PRL output were most likely to be a result of altered inhibition by hypothalamic dopamine, since it was tonic inhibition by this factor which primarily regulates PRL secretion (Lieberman, Maurer, Claude, Wiklund, Wertz, Gorski, 1981, Freeman *et al.*, 2000). Whilst vitamin E confirmed to have an effect on the monoamine metabolism in rat brain and vitamin E deficiency may reduce the dopamine levels in the brain stem(Adachi *et al.*, 1999).Thence, the concentration of PRL increased at low levels vitamin E and may cause abrupt increases of estradiol. As for the decrease of PRL content when vitamin E supplementation exceeded 20 mg/kg, that could be related to the increase of dopamine content in the brain which inhibited the synthesis and secretion of PRL.

In the current study, we confirmed that the supplementation of dietary vitamin E could improve the concentration of reproductive hormones in serum and the expression of reproductive hormone receptor genes in the ovary. Furthermore, it was observed that the appropriate level of vitamin E for reproductive hormone concentration tested in this study was 80mg/kg, which was consistent with the result of Lin's finding that 80mg/kg vitamin E was the most appropriate content for egg production, hatchability and fertility (Lin *et al.*, 2004). Plasma gonadotropin concentration was supposed to present strong correlations with gonadotropin genes in pituitary and gonadotropin receptor genes in the ovary (Brooks *et al.*, 1992). It was demonstrated that the effect of FSH on the ovary was mainly exerted by binding to the specific receptor of the granulosa cell membrane, *FSHR* (Lu *et al.*, 2009). In the current study, we found that the expression patterns of reproductive hormone receptor genes (*FSHR*, *ESR1*, *LHR* and *PRLR*) in the ovary were similar to the reproductive hormone expression patterns.

Additionally, *PRLR* mRNA expressed relatively lower in the ovary compared with other hormones. Therefore, the release of PRL in the pituitary could be inhibited by a higher concentration of E2. Interestingly, all hormones, but PRL, were observed to show a decreasing trend above 80 mg/kg vitamin E supplementation. This result could be explained as the negative feedback existing in hypothalamic-pituitary-gonadalaxis. Once the concentration of reproductive hormones in serum exceeds the normal level, a regulation of negative feedback in the hypothalamic- pituitary-gonadal axis would inhibit the origin of reproductive hormones. Moreover, high dose of vitamin E supplementation may reduce the cholesterol content for the synthesis of estradiol, which could further reduce the synthesis and release FSH and LH in the pituitary.

Dicer, as a ribonuclease, was found to be critical for reproduction. It was reported that suppressing the expression of *Dicer* could reduce the maturation rate of oocyte and suppression of gonadotropin synthesis (Wang *et al.*, 2015). As shown in the results (Figure 2, 3), the expression of *FSHR*, *ESR1* and *LHR* increased with the increasing expression of *Dicer* at 40-80 mg/kg dose of vitamin E supplementation in the ovary. Yu's view rests on the result that joint comparisons revealed 23 upregulated and 21 downregulated miRNAs in laying stage and confirmed miRNAs involved in follicular atresia directly (Yu *et al.*, 2016). Since miRNAs participate in follicular atresia and development, the expression of *Dicer* in the different stage of follicles should be detected. The relative mRNA expression of *Dicer* in the ovary, SWF, LWF and SYF were detected in this study. Compared with other tissues, lower mRNA expression of *Dicer* was observed in LWF with no vitamin E supplementation. This result was supposed to be associated with the different physiological changes, mainly protein and steroid accumulation, occurred in the ovary and follicles in different stages. As described in Guan's study, the main changes presented in pre-hierarchical follicle development were yolk accumulation, granulosa cell and membrane cell proliferation and differentiation(Guan *et al.*, 2015). However, the specific mechanism of *Dicer* expressed a decrease in LWF and needs to be clarified further. In this study, the relative mRNA expression of *Dicer* in the ovary increased at the dose of 40 mg/kg of vitamin E, whereas in SWF, LWF and SYF, Dicer mRNA expression–increased at the dose of 80 mg/kg of vitamin E supplementation. It was speculated that the differential expression of *Dicer* in the ovary and follicles affected by different doses of dietary vitamin E supplementation may be due to vitamin E sensitivity and protein expression diversity of ovary and follicles in different developmental stages.

ACKNOWLEDGEMENTS

This work was funded by the National Natural Science Foundation of China (Nos. 31172181 and 31660663) and the National Waterfowl Industrial Technology System (No. CARS-43-4 and 2016YFD0500510). We are grateful to the animals provided by Xingguo Grey Goose Research Base and research facility provided by the Institute of Animal Genetics and Breeding, Sichuan Agricultural University and all the volunteers who participatedin the study.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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