



## Age-related changes of yolk precursor formation in the liver of laying hens<sup>\*</sup>

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Received Jan. 26, 2017; Revision accepted May 9, 2017; Crosschecked Apr. 19, 2018

**Abstract:** A rapid decline in egg production of laying hens begins after 480 d of age. Such a rapid decrease results predominantly from the ovarian aging, accompanied by endocrine changes, decreased yolk synthesis and accumulation, and the reduction in follicles selected into the preovulatory hierarchy. In this study, hens at 90, 150, 280, and 580 d old (D90, D150, D280, and D580, respectively) were compared for yolk precursor formation in the liver to elucidate effects of aging on laying performance. The results showed that liver lipid synthesis increased remarkably in hens from D90 to D150, but decreased sharply at D580 as indicated by the changes in triglyceride (TG) levels. This result was consistent with the age-related changes of the laying performance. The levels of liver antioxidants and total antioxidant capacity decreased significantly in D580 hens and the methane dicarboxylic aldehyde in D580 hens was much higher than that at other stages. The serum 17 $\beta$ -estradiol level increased from D90 to D280, but decreased at D580 ( $P < 0.05$ ). The expression of estrogen receptor  $\alpha$  and  $\beta$  mRNAs in the liver displayed similar changes to the serum 17 $\beta$ -estradiol in D580 hens. Expressions of the genes related to yolk precursor formation and enzymes responsible for fat acid synthesis were all decreased in D580 hens. These results indicated that decreased yolk precursor formation in the liver of the aged hens resulted from concomitant decreases of serum 17 $\beta$ -estradiol level, transcription levels of estrogen receptors and critical genes involved in yolk precursor synthesis, and liver antioxidant status.

**Key words:** Lipid metabolism; Yolk precursor formation; 17 $\beta$ -Estradiol; Antioxidant; Hen  
<https://doi.org/10.1631/jzus.B1700054>

**CLC number:** S852.2

### 1 Introduction

Poultry eggs are the valuable sources of dietary proteins, lipids, amino acids, and minerals for human beings. After the onset of egg production, the maximal laying rate of commercial hens could reach more than 95% at the age around 200 d. However, there is a fast decline in egg production after the hens reach 480 d of age (Joyner et al., 1987). The decline of egg production in aged hens resulted predominantly from the ovarian aging, accompanied with endocrine changes (Buyuk et al., 2010), decreased yolk synthe-

sis and accumulation (Zakaria et al., 1983), and the reduction in number of follicles selected into the preovulatory hierarchy (Lillpers and Wilhelmson, 1993).

Reproductive aging in mammals includes progressive reduction in ovarian follicular reserve with decreased oocyte quality (Ishii et al., 2012; Ben-Meir et al., 2015). Unlike mammals, chicken eggs contain large stores of egg yolk proteins that consist of vitellogenin (VTG) and apolipoprotein (Apo). The development of chicken follicles is accompanied with absorption and deposition of a large amount of yolk precursors. Kim et al. (2014) found that egg weight, egg shell breaking strength, or Haugh unit decreased as the hens aged. However, there is very little information available about the influence of aging on yolk precursor formation in the laying hens.

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<sup>\*</sup> Project supported by the National Natural Science Foundation of China (Nos. 31472160 and 31272525)

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During egg production, large quantities of yolk precursors (triglyceride (TG), phospholipids, and cholesterol) are synthesized in the liver predominantly under the influence of estrogens (Johnson, 2015). Several age-related changes have been demonstrated including a decrease in the volume of hepatocytes and changes in hepatocyte nuclear morphology (Hoare et al., 2010). Nonetheless, age-related changes in the antioxidative capacity of the chicken liver, one of the most important factors that influence liver function, have not yet been described thoroughly. In normal cells, there is a major antioxidant defense system to scavenge reactive oxygen species (ROS), and thus to attenuate oxidative stress damage. However, the balance between ROS generation and antioxidant systems can be disrupted because of gradually decreased levels of antioxidants during the aging process (Tong et al., 2015). From these studies, we assume that the decrease of antioxidant defense function of the liver is responsible for the reduction of laying performance of the aged hens.

The yolk formation and deposition in hens depend largely on the function of the liver-blood-ovary axis under complex regulation of diverse hormones especially estrogens (Nagahama, 1994; Cohen and Smith, 2014; Yilmaz et al., 2015). For example, the expression of very low-density apolipoprotein II (ApoVLDLII) displays a strictly estrogen-dependent pattern in hens (Li et al., 2014). After binding to their receptors, estrogens exert their physiological functions in the target organs, such as liver and reproductive organs (Hall et al., 2001). Although three estrogen receptors (ERs), i.e. *ER- $\alpha$* , *ER- $\beta$* , and G protein-coupled receptor 30 (*GPR30*), were all expressed in the chicken liver, Li et al. (2014) demonstrated that estrogen-mediated stimulation of apolipoprotein B (*ApoB*) and vitellogenin II (*VTGII*) genes appeared to be predominantly through the *ER- $\alpha$*  signaling pathway. Decreased estrogen production is inevitably accompanied with aging in the female, resulting in reduced fecundity. In the aged hens, increased yolk deposition after follicle stimulating hormone (FSH) treatment may be caused by elevated serum 17 $\beta$ -estradiol ( $E_2$ ) level (Palmer and Bahr, 1992). However, there is little information available regarding the changes in serum  $E_2$  related with variation in yolk precursor formation in the aging process.

In this study, hens at different stages were compared for the liver yolk precursor formation in order to

elucidate the effects of aging on laying performance. Biochemical parameters related to yolk synthesis and hormonal regulation in livers were determined. The results will expand the knowledge on yolk formation which should be beneficial for poultry production.

## 2 Materials and methods

### 2.1 Animals

Hyline brown hens were raised in a local commercial farm and subjected to conventional feeding management conditions. Sample collection was performed from hens of 90, 150, 280, and 580 d old (D90, D150, D280, and D580, respectively) that reflected four different laying stages, i.e. before laying, early laying, peak laying, and later laying periods. Blood samples were collected via the wing vein between 10 and 12 h before the next oviposition. Chickens were slaughtered by decapitation and the livers and ovaries without prehierarchal and preovulatory follicles were immediately snap-frozen in liquid nitrogen for analysis of biochemical parameters and RNA extraction.

### 2.2 Biochemical analysis

To measure biochemical parameters, 0.3 g liver or ovarian tissues were homogenized in 2.7 ml phosphate buffer saline (PBS). The protein concentration in the tissue homogenates was determined using a Total Protein Assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

#### 2.2.1 TG and total cholesterol

The concentrations of TG and total cholesterol (T-CHO) in serum, liver, and ovary were measured using respective commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

#### 2.2.2 Oxidative stress parameters

The activity of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), glutathione *S*-transferase (GSH-ST), catalase (CAT), and total antioxidant capacity (T-AOC), and the concentrations of glutathione (GSH), hydrogen peroxide ( $H_2O_2$ ), and methane dicarboxylic aldehyde (MDA) were measured according to the manufacturer's instructions with kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

### 2.2.3 Serum E<sub>2</sub>

Serum E<sub>2</sub> was measured with an IMMULITE-2000 Estradiol kit (Siemens Medical Solutions Diagnostics, Glyn Rhonwy, UK) according to the manufacturer's instructions. There were ten samples with three repetitions in each group.

### 2.3 RNA isolation and real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from livers using an ultraturax homogeniser in TRIzol reagent (TaKaRa Bio Inc., Shiga, Japan). The quantity of RNA was then assessed using a NanoDrop ultraviolet-visible (UV-Vis) spectrophotometer. The complementary DNA (cDNA) was generated from 2 µg of RNA using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, San Jose, CA, USA). Qualitative PCR (qPCR) was carried out on 2 µl cDNA templates.

PCR products were visualized in 1.5% (15 g/L) agarose gel stained with ethidium bromide.

### 2.4 RNA isolation and qPCR

The qPCR was carried out on an ABI 7500HT Real-Time PCR machine (Applied Biosystems, CA, USA) with 2 µl cDNA template, 400 nmol/L of each of the gene-specific forward and reverse primers (Table 1), 0.4 µl Rox reference dye II, 10 µl SYBR Premix Ex Taq (TaKaRa, Bio Inc., Shiga, Japan) and 6.8 µl water in a total volume of 20 µl. qPCR conditions were as follows: 95 °C for 10 min, and then 40 cycles of 95 °C for 30 s, 60 °C for 34 s, and 72 °C for 30 s. All samples were measured in triplicate and the experiments were performed twice. The relative abundance of each transcript was normalized with *β-actin*. Gene expression was calculated using the 2<sup>-ΔΔCt</sup> method and results are expressed as fold change in expression over D90 pullets.

**Table 1 Primers for PCR analysis**

Gene	Accession No.	Primer sequence (5'→3')	Product size (bp)
<i>ER-α</i>	NM_205183.2	Forward: TAGTTCGCTCTACGACCTCT Reverse: AGTTGGTTTCGGTTCTCCTCTT	106
<i>ER-β</i>	NM_204794.2	Forward: AAAGAACAGAAACCCCATTCAG Reverse: GCACAGAGGGACATTTTGATT	82
<i>GPR30</i>	NM_001162405.1	Forward: AGGTCCAAGGATGTGCGCTGA Reverse: GTCGTAAGACCACGGCGGGA	156
<i>VTGII</i>	NM_001031276	Forward: TTGCAAGCTGATGAACACACAC Reverse: GATTGCTTCATCTGCCAGGTC	192
<i>ApoVLDLII</i>	NM_205483	Forward: CTTAGCACCCTGTCCCTGAAGT Reverse: TGCATCAGGGATGACCAGC	81
<i>ApoB</i>	NM_001044633.1	Forward: ACACCTCGGGCTATTGGA Reverse: TGCCTGTATGGCTGCTTT	129
<i>SREBP</i>	NM_204126.2	Forward: AGGAGCCACAATGAAGACCG Reverse: GTACTGTGGCCAGGATGGTC	110
<i>FAS</i>	NM_205155.1	Forward: GCTAAGATGGCATTGCACGG Reverse: TCCATTTCAGTTCCAGACGGC	135
<i>ACC</i>	NM_205505.1	Forward: GGCTGGGTTGAGCGACTAAT Reverse: TCCACATTGGGTGGAATCCG	107
<i>MTP</i>	XM_420662.2	Forward: GTTCTGAAGGACATGCGTGC Reverse: GATGTCTAGGCCGTACGTGG	120
<i>ACLY</i>	NM_001030540.1	Forward: GGGCCACAAAGAAATCTTGA Reverse: CAGCAATAATGGCAATGGTG	167
<i>SCD</i>	NM_204890	Forward: GACCCTCACAATGCTATGC Reverse: CACCACTTTGTCAGCCTTTA	129
<i>MALI</i>	AF408407	Forward: AGTGCCCTACCTGTGATGTTG Reverse: GGCTTGACCTCTGATTCTCT	101
<i>PPARα</i>	NM_001001464.1	Forward: AGGCCAAGTTGAAAGCAGAA Reverse: TTTCCCTGCAAGGATGACTC	155
<i>PPARγ</i>	NM_001001460.1	Forward: TGACAGGAAAGACGACAGACA Reverse: CTCCACAGAGCGAAACTGAC	164
<i>β-actin</i>	NM_205518	Forward: ACACCCACACCCCTGTGATGAA Reverse: TGCTGCTGACACCTTACCATTTC	136

*ER*: estrogen receptor; *GPR30*: G protein-coupled receptor 30; *VTGII*: vitellogenin II; *ApoVLDLII*: very low-density apolipoprotein II; *ApoB*: apolipoprotein B; *SREBP*: sterol regulatory element-binding protein; *FAS*: fatty acid synthase; *ACC*: acetyl coenzyme A carboxylase; *MTP*: microsomal triglyceride transport protein; *ACLY*: adenosine triphosphate citrate lyase; *SCD*: stearoyl-CoA desaturase; *MALI*: malic enzyme; *PPARα*: peroxisome proliferator-activated α; *PPARγ*: peroxisome proliferator-activated γ

## 2.5 Statistical analysis

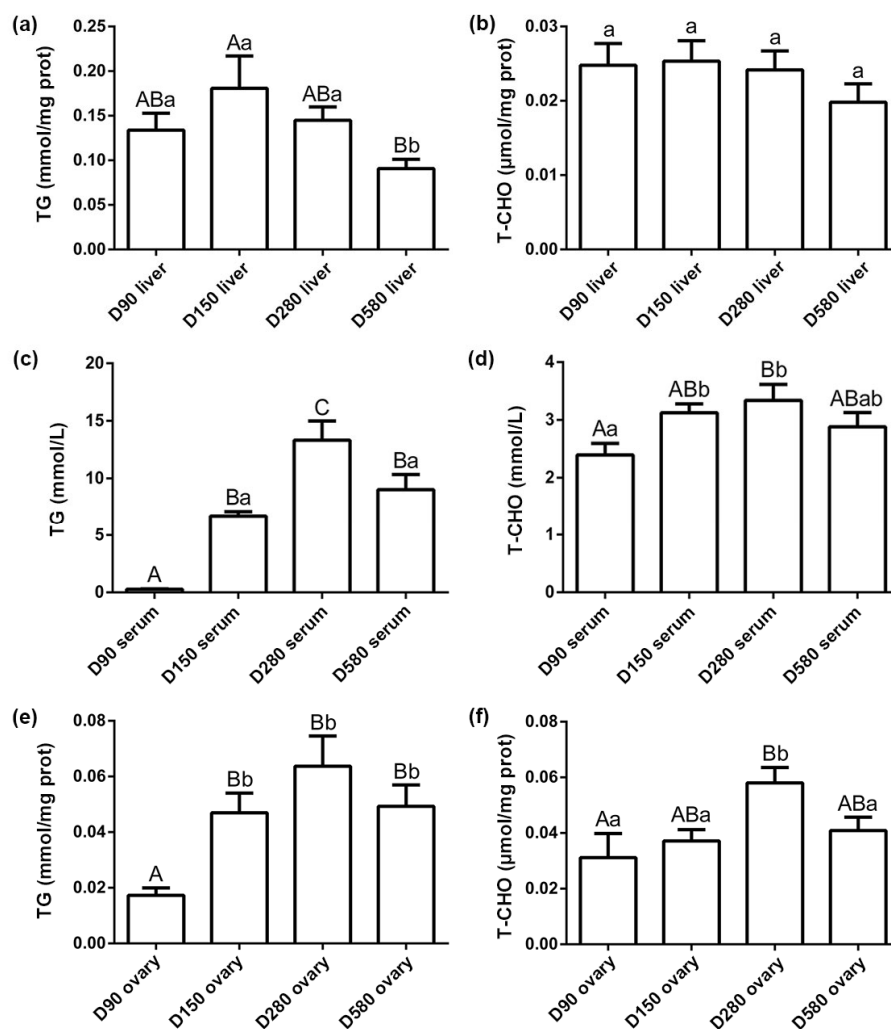
All experimental data are presented as mean± standard error (SE). Analyses were performed with a one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA).  $P<0.05$  was considered as a statistically significant difference.

## 3 Results

### 3.1 TG levels in the liver and serum

Follicles with diameter >12 mm were considered as hierarchical follicles in this study. Almost all of the

D150 hens (9 in 10) had 6 yolk-filled follicles in the hierarchy, while the fifth largest follicles in D580 hens were smaller than 12 mm in diameter. TG levels in the liver, serum, and ovary began to rise from D90 to D150 as the hens started to lay eggs and reached the highest levels in the D150 or D280 hens, but decreased in D580 aged hens with decreased laying performance (Figs. 1a, 1c, and 1e). There was no significant difference in liver T-CHO levels in the four stages (Fig. 1b). Serum T-CHO level increased from D90 to D150 and remained at higher levels in D280 and D580 hens (Fig. 1d). In the ovary, T-CHO level in D280 hens was significantly higher than that in the others ( $P<0.05$ ; Fig. 1f).



**Fig. 1** Changes of TG and T-CHO levels in the liver, serum, and ovary in hens of different ages

TG (a, c, e) and T-CHO (b, d, f) levels were determined in the liver (a, b), serum (c, d), and ovary (e, f) of hens aged 90, 150, 280, and 580 d. Data were expressed as the mean±SE ( $n=10$ ). Different uppercase and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively. prot: protein

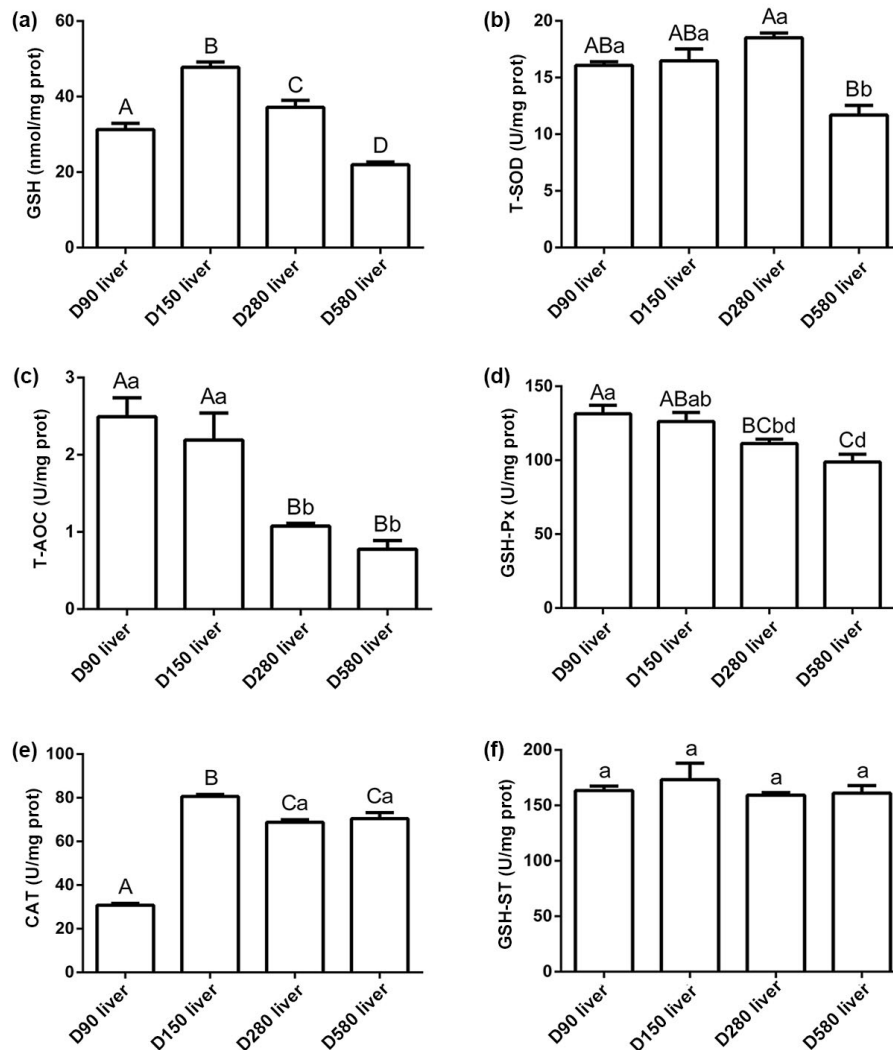
### 3.2 Antioxidant capacity of the liver

Levels of GSH and T-SOD in D580 hen livers were significantly lower than those in other groups ( $P<0.05$ ; Figs. 2a and 2b). No significant difference was detected in T-SOD levels among D90, D150, and D280 livers. T-AOC and GSH-Px levels in D580 livers were lower than those in D90 and D150 livers ( $P<0.05$ ; Figs. 2c and 2d). A significant increase was observed in both liver GSH and CAT levels associated with sexual maturation (Figs. 2a and 2e). There was no significant difference in GSH-ST activity among the four groups (Fig. 2f). MDA level in D580 hen liver was significantly higher than that in other groups ( $P<0.01$ ), but there was no significant difference

among D90, D150, and D280 hen livers (Fig. 3a).  $H_2O_2$  level in D580 hen liver was higher than that in D90 and D280 hen livers ( $P<0.05$ ; Fig. 3b). These results remarkably indicated that decreased GSH, T-SOD, T-AOC, and GSH-Px levels, and increased MDA and  $H_2O_2$  levels in livers appeared during the aging process.

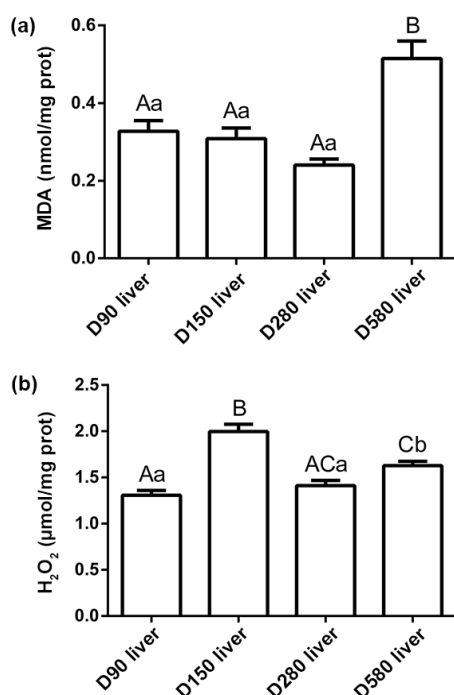
### 3.3 Serum $E_2$ and mRNA expression of liver $ER-\alpha$ and $ER-\beta$

Serum  $E_2$  level began to rise from D90 to D150 as the hens started to lay eggs and reached the highest levels at the peak laying period (D280), but decreased significantly at the later laying period (D580) with decreased laying performance ( $P<0.05$ ; Fig. 4).



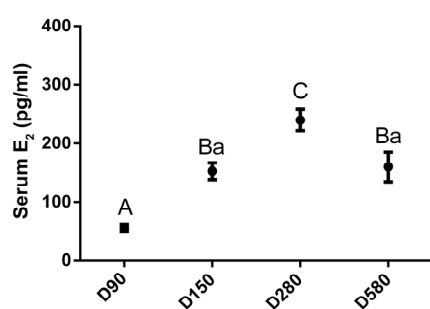
**Fig. 2** Liver antioxidant levels in hens of different ages

Levels of GSH (a), T-SOD (b), T-AOC (c), GSH-Px (d), CAT (e), and GSH-ST (f) were determined in hens aged 90, 150, 280, and 580 d. Data were expressed as the mean $\pm$ SE ( $n=10$ ). Different uppercase and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively. prot: protein



**Fig. 3** Liver MDA and H<sub>2</sub>O<sub>2</sub> levels in hens of different ages

Levels of MDA (a) and H<sub>2</sub>O<sub>2</sub> (b) were determined in hens aged 90, 150, 280, and 580 d. Data were expressed as the mean±SE ( $n=10$ ). Different uppercase and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively

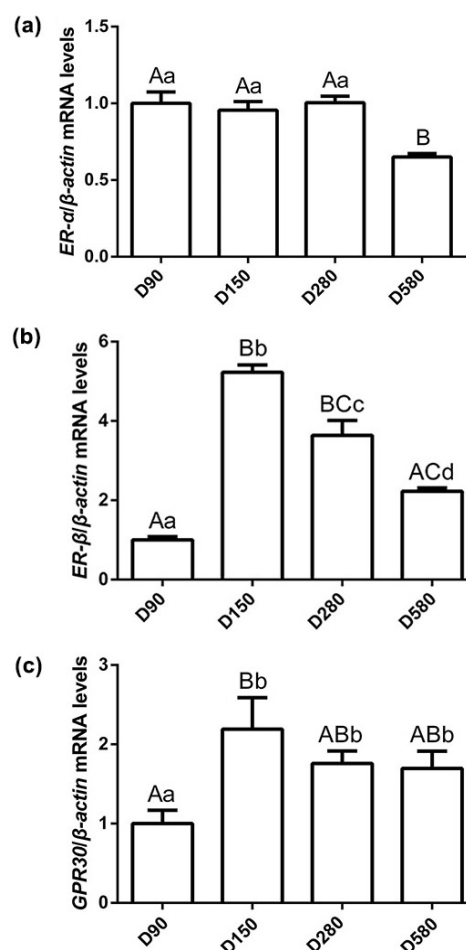


**Fig. 4** Levels of serum E<sub>2</sub> in hens of different ages

Data were expressed as mean±SE ( $n=10$ ). Different uppercase and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively

The expression of *ER-α* mRNA was significantly lower in D580 hen livers than in other groups ( $P<0.01$ ; Fig. 5a). For *ER-β* mRNA expression, there was a sharp rise from D90 to D150 ( $P<0.01$ ), and then a significant decline appeared from D150 to D280 and D580 ( $P<0.05$ ; Fig. 5b). There was no considerable

difference in the expression of *ER-α* mRNAs among D90, D150, and D280 livers, while the expression of *GPR30* mRNA in D90 hen liver was lower than that in other groups. No significant difference was found in the *GPR30* mRNA levels among D150, D280, and D580 hen livers (Fig. 5c).

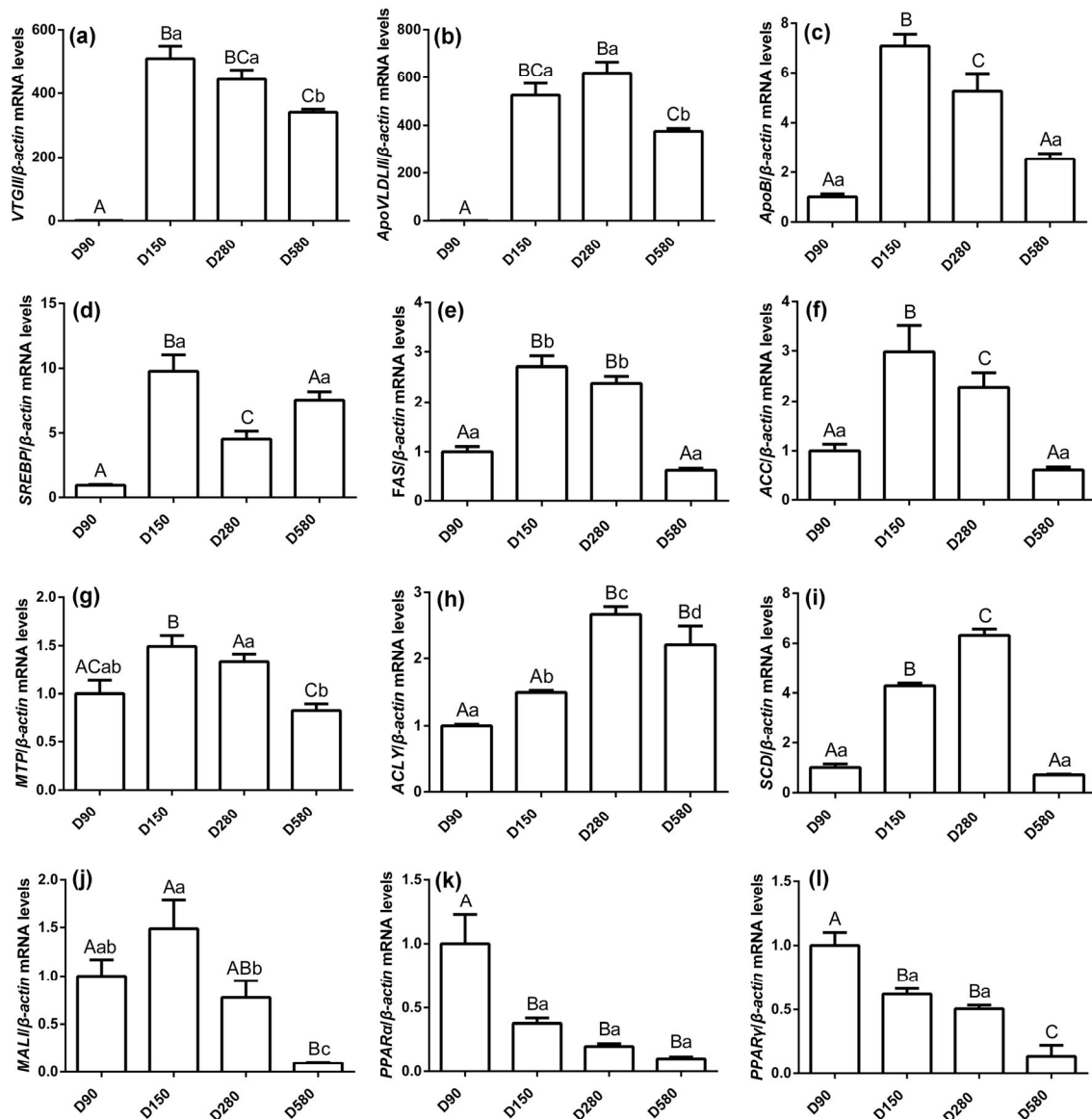


**Fig. 5** Expression of *ER-α* (a), *ER-β* (b), and *GPR30* (c) mRNAs in liver of hens of different ages

Data were expressed as mean±SE ( $n=10$ ). The relative abundance of each transcript was normalized to *β-actin* and expressed as fold changes over D90 pullets. Different uppercase and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively

### 3.4 Expression of mRNAs related to yolk precursor synthesis in the liver

The expression of *VTGII*, *ApoVLDLII*, and *ApoB* mRNAs in hen livers increased markedly from D90 to D150 ( $P<0.01$ ) and remained at higher levels at D280 while it decreased sharply at D580 ( $P<0.01$ ; Figs. 6a–6c).



**Fig. 6** Transcription levels of genes related to yolk precursor synthesis in hens of different ages

Transcription levels of *VTGII* (a), *ApoVLDLII* (b), *ApoB* (c), *SREBP* (d), *FAS* (e), *ACC* (f), *MTP* (g), *ACLY* (h), *SCD* (i), *MALI* (j), *PPARα* (k), and *PPARγ* (l) in livers of hens aged 90, 150, 280, and 580 d. Data were expressed as the mean±SE ( $n=10$ ). The relative abundance of each transcript was normalized to  $\beta$ -actin and expressed as fold changes over D90 pullets. Different capital and lowercase letters indicate a very significant difference ( $P<0.01$ ) and significant difference ( $P<0.05$ ), respectively

Expression of the sterol regulatory element-binding protein (*SREBP*) mRNA in D280 hen livers was higher than that in D90 livers ( $P<0.01$ ), but lower than that in the D150 and D580 livers ( $P<0.01$ ; Fig. 6d). The mRNA levels of fatty acid synthase (*FAS*), acetyl coenzyme A carboxylase (*ACC*), microsomal triglyceride transport protein (*MTP*), adenosine triphosphate citrate lyase (*ACLY*), stearoyl-CoA deasturase (*SCD*), and malic enzyme (*MALI*) in livers increased along

with the elevated egg production from D90 to D150 and D280, then decreased at D580 with the reduced egg production. Interestingly, *FAS*, *ACC*, *MTP*, and *MALI* expression reached the maximal level at D150 while *ACLY* and *SCD* reached peak levels at D280 in four different laying stages (Figs. 6e–6j). Contrary to these changing trends, there was a gradual decline in the expression of the peroxisome proliferator-activated  $\alpha$  (*PPARα*) and peroxisome proliferator-

activated  $\gamma$  (*PPAR $\gamma$* ) mRNAs from D90 to D580 (Figs. 6k and 6l). These findings demonstrated that transcription levels of the critical genes in yolk precursor synthesis changed during the aging process.

#### 4 Discussion

In the highly productive laying hens, a dramatic decrease in egg production appears at the end of the first-year laying cycle. Therefore, it is necessary to understand the mechanisms of deterioration of the laying performance during the aging process. In the present study, we investigated the effects of aging on yolk precursor formation in the liver via comparing TG and T-CHO levels in the liver-ovary axis, antioxidant capacity of the liver, serum  $E_2$  levels, and transcription levels of the *ERs* and genes related to yolk precursor synthesis in different laying stages. Our results first validate that the capacity of yolk lipid formation in hens may decrease with increasing age as a consequence of changes in liver antioxidant levels, serum  $E_2$  levels, and transcription of the critical genes involved in yolk precursor synthesis.

In the ovary of sexually mature hens, there are numerous slow-growing follicles approximately 2–6 mm in diameter that contain small amounts of a protein-rich white yolk. One follicle is selected per day from 6–8 mm prehierarchal follicles into preovulatory follicles, followed by a final rapid growth. During the final growth phase, a large amount of VTG and ApoB can be synthesized in the liver and then transported via blood circulation to the ovaries for deposition into the follicles. Counts of the largest yolky follicles provide a reliable indicator of the reproductive status in the hens (Bahr and Johnson, 1984). Our results showed that the number of hierarchical follicles and TG levels in the liver and serum of the aged hens were lower than those of the young hens, indicating that there was a decline in the ability of yolk precursor synthesis during the aging process.

Accumulating evidence suggests that the antioxidant capacity would be degenerated because of declining antioxidant levels in the aging process. As a consequence, the organs will lose normal physiological function (Ben-Meir et al., 2015; Okudan and Belviranlı, 2016). In rats, SOD, CAT, and GSH levels in the cerebella tissue from an 18-month-old group

were significantly lower than those from 6- and 12-month-old groups (Subramanian and James, 2010). Consistent with this comparison, in the present study, we found that the levels of GSH, T-SOD, GSH-Px, and T-AOC in liver tissue from D580 hens were lower than those from younger hens while the MDA level was higher than that in younger hens.

In the oviparous vertebrates, VTGII and ApoB are synthesized by the liver in response to estrogens (Verderame and Limatola, 2010; Hess et al., 2011; Yilmaz et al., 2015). Our results showed that the serum  $E_2$  level of D280 hens was significantly higher than that in hens at other stages, and these findings are in line with a previous report that in the domestic Turkey hens the serum  $E_2$  level during the laying period was higher than that in incubating or out-of-lay hens (Khalil et al., 2009). These data also concur with a previous report that the plasma  $E_2$  level in 17-week-old hens was higher than that in 13-week-old hens (Reddy et al., 2002).

The activation of  $E_2$  on *VTG* gene expression is mediated principally through a genomic mechanism driven by specific ERs. In the rainbow trout, both ER- $\alpha$  and ER- $\beta$  were proved to mediate the synthesis of VTG (Leaños-Castañeda and van der Kraak, 2007). Our previous study demonstrated that estrogen-mediated stimulation of *ApoB* and *VTG* genes was predominantly through ER- $\alpha$  in the chicken hepatocytes (Li et al., 2014) and  $E_2$  directly bound to GPR30 to activate the epidermal growth factor receptor (EGFR)/Akt/ $\beta$ -catenin pathway, thereby mediating estrogen-induced proliferation of chicken primordial germ cells (Ge et al., 2012). *ER- $\alpha$*  had a significant increase in the reproductive period in the Chinese *Alligator sinensis* (Zhang et al., 2016). In the present study we observed that the expression of *ER- $\alpha$*  and *ER- $\beta$*  mRNAs decreased during the aging process. Meanwhile, levels of *ER- $\beta$*  and *GPR30* mRNAs in D90 pullets were remarkably lower than those in the adult hens. There have been similar findings that the transcription level of *ER- $\beta$*  mRNA was significantly higher in laying hens compared with pullets (Li et al., 2014).

After synthesis in the liver, ApoB is packaged into VLDL particles for transportation while VTG is secreted into blood (Ratna et al., 2016). Changes in hepatic lipogenic gene expression in the female chickens resulted in a shift of fatty acid synthesis



toward fatty acid  $\beta$ -oxidation (Bai et al., 2015). In this study, levels of *VTGII*, *ApoVLDLII*, and *ApoB* mRNAs in the liver of D90 pullets were lower than those in the laying hens. In addition, the levels of *VTGII*, *ApoVLDLII*, and *ApoB* mRNAs in D580 hen livers were markedly lower than in D150 and D280 hen livers. These results were in accordance with those where the levels of *ApoB* and *ApoVLDLII* mRNAs were increased following estrogen release after laying (Claire D'Andre et al., 2013; Li et al., 2015). We also observed that the expressions of genes related to lipogenesis (*ACC*, *FAS*, *SREBP*, *SCD*, *ACLY*), lipolysis (*PPAR $\gamma$* ), and lipid transport (*MTP*) were all decreased along with the aging process. These results were consistent with the changes that TG and T-CHO levels in the liver tissue of D580 hens were detected lower than those in D150 and D280 hens.

In summary, this study demonstrated that in hens, the ability of yolk precursor formation is decreased in the aging process as a result of decreased serum  $E_2$  level, transcription of *ER- $\alpha$* , *ER- $\beta$* , and critical genes (*VTGII*, *ApoB*, *ApoVLDLII*, *FAS*, *ACC*, *MTP*, *ACLY*, *SCD*, *MALI*, and *PPAR $\gamma$* ) in yolk precursor synthesis, and liver antioxidant levels.

### Acknowledgements

We sincerely thank Chang-quan GUO, Guang LIU, and Rong-xia LI (College of Animal Sciences, Zhejiang University, Hangzhou, China) for their help in the experiments.

### Compliance with ethics guidelines

Xing-ting LIU, Xin LIN, Yu-ling MI, Wei-dong ZENG, and Cai-qiao ZHANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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## 中文概要

**题目:** 不同产蛋阶段蛋鸡卵黄前体物质生成的变化研究

**目的:** 比较不同日龄蛋鸡血清雌激素的水平、肝脏雌激素受体基因和卵黄前体物生成相关基因表达量、“肝脏-血液-卵巢轴”中甘油三酯和胆固醇的含量和肝脏抗氧化能力的动态变化,揭示蛋鸡卵黄前体物生成随着年龄增长的变化规律。

**创新点:** 首次发现在蛋鸡衰老过程中雌激素作用衰退以及肝脏抗氧化能力降低致使肝脏中卵黄前体物合成下降,最终导致蛋鸡的产蛋性能下降。

**方法:** 取 90、150、280 和 580 日龄蛋鸡各 10 只,检测血清中雌激素水平、肝脏雌激素受体基因表达水平、肝脏中卵黄前体物质合成和脂质代谢相关基因表达水平、“肝脏-血液-卵巢轴”中甘油三酯和胆固醇的含量及肝脏抗氧化能力,揭示蛋鸡卵黄前体物生成在衰老过程中的变化规律。

**结论:** 在蛋鸡衰老过程中,血清雌激素水平、肝脏雌激素受体基因和卵黄前体物质合成相关基因表达量降低,肝脏抗氧化能力降低,引起肝脏合成甘油三酯的能力下降,最终导致卵黄前体物质合成能力降低。

**关键词:** 脂质代谢; 卵黄前体物质; 雌激素; 抗氧化物质; 蛋鸡