High levels of vitamin E affect retinol binding protein but not CYP26A1 in liver and hepatocytes from laying hens

X. D. Zhou, X. F. Dong,[1] J. M. Tong, P. Xu, and Z. M. Wang

State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China

ABSTRACT This study was conducted to determine the influence of vitamin E on the retinol binding protein (RBP) and cytochrome p450 family 26 subfamily A polypeptide 1 (CYP26A1), which are specific transporters and catabolic enzymes of vitamin A, respectively. In the in vivo experiments, a total of 450 laying hens was fed 5 levels of vitamin E (0, 20, 80, 320, and 1,280 IU/kg of feed) supplementation. For the in vitro assays, hepatocytes from laying hens were cultured in 4 levels of α-tocopherol (0, 10, 50, and 100 μ*M*). High dietary vitamin E increased the concentration of vitamin A in liver ($P < 0.05$). The RBP and its mRNA expression in liver and hepatocytes were markedly inhibited by dietary vitamin E (320 and 1,280 IU/kg) and α-tocopherol (100 μ*M*) in culture medium ($P < 0.05$). However, CYP26A1 and its mRNA expression were not affected by vitamin E in both liver and hepatocytes ($P > 0.05$). The results indicate that excessive vitamin E could increase the concentration of vitamin A in liver by inhibiting RBP synthesis in hepatocytes.

Key words: cytochrome p450 family 26 subfamily A polypeptide 1, hepatocyte, laying hen, retinol binding protein, vitamin E

2012 Poultry Science 91:1135–1141
http://dx.doi.org/10.3382/ps.2011-01914

INTRODUCTION

Vitamins E and A are essential fat-soluble vitamins that play important roles in several similar basic biological functions, such as reproduction and immune system regulation (Sheridan and Beck, 2008; Long et al., 2011). Previous studies demonstrated that with the elevation of dietary vitamin E (0–1,000 IU/kg of diet) in laying hens, the level of vitamin A in liver significantly increased 16.3-folds, while it decreased by 27.4% in yolk and remained unchanged in plasma (Sklan, 1983; Sünder et al., 1999; Sünder and Flachowsky, 2001). Napoli et al. (1984) pointed out that 100 μ*M* tocopherol inhibited retinol hydrolase activity by about 50% in rat liver. This may be one reason why dietary vitamin E increased vitamin A in mammalian liver. However, retinol hydrolase has not been identified in poultry.

Recently, researchers have gained more information about the transport and catabolism of vitamin A. Zanotti et al. (2001) found that retinol binding protein (**RBP**) was a specific transport protein of vitamin A in the plasma of chicken, and they found that the liver was the major organ of RBP synthesis and secretion. White et al. (1997) were the first to identify the cytochrome P450 family 26 subfamily A polypeptide 1 (**CYP26A1**), which belongs to the cytochrome P450 (**CYP450**) super family, involved in catabolism of vitamin A. This CYP26A1 can oxidize retinoic acid into its inactive forms, 4-oxo-retinoic acid and 4-hydroxy-retinoic acid, which are excreted through the digestive system (Ray et al., 1997; Swindell et al., 1999; Lampen et al., 2001; Perlmann, 2002). However, no study provides insight into the possibility that vitamin E might influence vitamin A by regulating RBP and CYP26A1. The current investigation was designed to elucidate the effects of vitamin E on increasing vitamin A concentrations in liver after dietary administration in vivo or their direct addition in vitro.

MATERIALS AND METHODS

Chemicals

DL-α-Tocopheryl acetate (purity 50%) and α-tocopherol (purity 96%) were purchased from Guobang Pharmaceutical Chemical Group Co. Ltd. (Zhejiang, China) and Sigma-Aldrich Co. (Shanghai, China), respectively.

Experimental Design

Experiment 1. This experiment was conducted to determine the effects of feeding a gradient of vitamin

Received October 5, 2011.
Accepted January 14, 2012.
[1]Corresponding author: xiaofangd1124@sina.com

Table 1. Ingredient composition and calculated concentration of nutrients of the basal diet

Ingredient	%	Nutrient	Amount
Corn	54.7	ME (MJ/kg)	11.50
Soybean meal	27.0	CP (%)	16.60
Limestone	9.0	Calcium (%)	3.72
Lard	4.0	Nonphytate phosphorus (%)	0.34
Salt	0.3	Methionine (%)	0.35
Vitamin/mineral premix[1]	5.0	Methionine + cysteine (%)	0.67

[1]Provided per kilogram of feed: vitamin A, 8,000 IU; vitamin D_3, 2,500 IU; vitamin K_3, 1.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.2 mg; choline chloride, 500 mg; folic acid, 2.3 mg; niacin, 40.2 mg; pantothenic acid, 14.4 mg; pyridoxine, 8.6 mg; riboflavin, 8.2 mg; thiamin, 4.6 mg; zinc, 70.0 mg; iron, 60.0 mg; manganese, 80.0 mg; copper, 8.0 mg; iodine, 0.5 mg; selenium, 0.3 mg; dicalcium phosphate 18 g.

E (DL-α-tocopheryl acetate) on concentrations of vitamin A in liver, yolk, and plasma of laying hens and RBP and CYP450 in tissues. The mRNA expression of *RBP* and *CYP26A1* were also detected in tissues. All experimental procedures were approved by the Beijing Administration Office of Laboratory Animals. Four hundred and 50 Jing Hong hens (a brown-egg laying hen), 24 wk of age, with similar BW (1.47 ± 0.10 kg) and laying rate (70 ± 5%) were randomly divided into 5 groups, which were assigned to one control group and 4 treatment groups. Each group had 6 replicates with 15 birds. Feed and water were supplied ad libitum, and the hens received 16 h of light/d throughout the course of this experiment. The control group was fed a diet based on corn-soybean meal that was formulated to fulfill all nutrient requirements for laying hens (NRC, 1994), but vitamin E was not included in the vitamin/mineral premix. The raw material composition and nutritive value of the diets are shown in Table 1. From this basal diet, 4 diets containing different concentrations of vitamin E (20, 80, 320, or 1,280 IU/kg of diet) were prepared. The analyzed concentration of vitamin E and A in each group is presented in Table 2. Prior to the feeding trial, all birds were fed the basal diet for 2 wk, and the experiment lasted for 8 wk.

Experiment 2. This experiment was carried out to investigate the influence of α-tocopherol on *RBP* and *CYP26A1* mRNA expression in hepatocytes from laying hens. The laying hens were fed vitamin E nonsupplemental diets, as described in experiment 1. The methods and materials used in hepatocyte isolation and culture procedures were based on the literature, with some modifications (Fraslin et al., 1992). The tubal vein, pancreaticoduodenal vein, and posterior mesenteric vein in the distal end were ligated before liver perfusion. The culture conditions were identical with normal hepatocytes (Sato et al., 2005). The medium was William's medium E (Sigma, Shanghai, China), containing 2 m*M* glutamine (Sigma), 100 U/mL of penicillin (Sigma), 100 μg/mL of streptomycin (Sigma), 1 μ*M* human insulin (Sigma), 1 μ*M* dexamethasone (Sigma), 10 μg/mL of human transferrin (Sigma), and 10 μg/mL of ascorbic acid (Sigma). The initial medium was replaced with α-tocopherol (0, 10, 50, and 100 μ*M*) supplemented William's medium E (serum-free) after 5 h in culture. The experiment was repeated 4 times.

Sample Collection and Assay

In experiment 1, egg production and egg weights were measured daily throughout the experimental period. Feed consumption and feed conversion ratio were measured in each week. Eggs in each replicate were collected and the shell hardness, Haugh units, yolk color, shell thickness, and albumen height were tested each 2 wk. Vitamin E and vitamin A in the diets were determined at the beginning of the experiment. At the end of the 4th and the 8th weeks, all egg yolks were collected, and one hen from each replicate was randomly selected and slaughtered to get liver and plasma samples to determine vitamin E and vitamin A using HPLC (e2695, Waters Alliance, Vaughn, WA) described by Sünder and Flachowsky (2001). Analysis of RBP in liver and plasma was carried out with commercial chicken ELISA kits (R and D Systems China Co. Ltd., Shanghai, China). The ELISA was performed according to the manufacturer's instructions and the description of Mao et al. (1996). Briefly, 5 g of liver samples were neutralized by adding 15 mL of 1 mol/L PBS (pH 7.4) and homogenized by grinders. Each specimen (liver homogenate or plasma) was centrifuged (3,000 × *g* for 20 min at 4°C), and supernatants were collected. The polystyrene microtiter plate was coated with anti-RBP antibody overnight at 4°C. Diluted RBP calibrator solution or

Table 2. Analyzed concentration of vitamin E and A in the experimental diets

	Vitamin E supplemental level[1] (IU/kg)				
Item	0	20	80	320	1,280
Vitamin E	4 ± 1	20 ± 1	83 ± 2	291 ± 42	1,068 ± 85
Vitamin A	7,739 ± 362	7,846 ± 549	7,713 ± 706	7,770 ± 347	7,991 ± 474

[1]Results are shown as means ± SE. Means and SE originating from 6 independent samples taken from each diet.

samples, antibody-enzyme conjugate, and *o*-phenylenediamine substrate were added separately to each well of the plate during each interval. The plate was incubated for 30 min at 37°C and washed 5 times with PBS containing 3.3 g/L of BSA. The plate was then agitated for 15 min at 37°C. The reaction was stopped with 50 μL of 2 mol/L solution of sulfuric acid, and the absorbance of the mixture was measured at 450 nm with a microplate reader (MK3; Thermo Scientific, Waltham, MA). Each liver and plasma sample was tested in duplicate. The hepatic microsome was prepared by differential velocity centrifugation (Rifkind et al., 1982), and the CYP450 was measured as Yang and Jiang (2006) described. Analysis of *RBP* and *CYP26A1* expression at the mRNA level was carried out by semiquantitative PCR according to the literature (Morris and Davila, 1996; Meadus, 2003). Total RNA was extracted from 100 mg of livers from laying hens with the total RNA isolation (TRIzol) reagent (Tiangen Biotech, Beijing, China), following the manufacturer's instructions. First-stand cDNA was synthesized with M-MLV reverse transcriptase from Promega (Fitchburg, WI), according to the manufacturer's instructions. To amplify the conserved region of *RBP*, *CYP26A1*, and *GAPDH* (housekeeping gene) cDNA, 3 pairs of primers were designed according to the conserved regions of *RBP*, *CYP26A1*, and *GAPDH* genes from *gallus* using the DNAssist 2.0 software (H. Patterton and S. Graves). The primers of *RBP* were L1 (5′-CTTCAATAACTGGGATGTCTG-3′) and L2 (5′-GGTCTATCTGCCTTTGTCTAAC-3′). The primers of *CYP26A1* were L1 (5′-ATGGAGCACACACAGGGTAAT-3′) and L2 (5′-GTTGAACTCGTCCTTGTCGGT-3′). The primers of *GAPDH* were L1 (5′-AGGGCACTGTCAAGGCTGAG-3′) and L2 (5′-ACGCTGGGATGATGTTCTGG-3′). The PCR products were first incubated at 94°C for 5 min, and then incubated by a stepped program (94°C, 40 s; 55°C for RBP, 54°C for *CYP26A1,* or 56°C for *GAPDH*, 30 s; 72°C, 40 s) for 27 cycles, and by an extension at 72°C for 5 min. The PCR products were about 240 and 423 bp long, respectively. The PCR products were separated on 2% agarose gels. The gel images were digitally captured with a Bio-Imaging system. Densitometry values were measured by the One-Dscan software (Scanalytics, Fairfax, VA). The semiquantitative PCR was repeated 3 times.

In experiment 2, cells were harvested 20 min after the addition of α-tocopherol to the medium (Dixon and Goodman, 1987). The lactate dehydrogenase activity (**LDH**) and total protein in the supernatant were determined with commercial diagnostic kits (Jiancheng Bio Co., Nanjing, China) and bicinchoninic acid assay (Beyotime Biotechnology Co., Haimen, China; Liu et al., 2004; Hao et al., 2010). The content of RBP was measured as described previously. Analysis of *RBP* and *CYP26A1* expression at the mRNA level was carried out by semiquantitative PCR as described in experiment 1, with the only modification that total RNA was extracted from the hepatocytes of laying hens.

Statistical Analysis

All data was analyzed using the one-way ANOVA (ANOVA), and means were compared by the Duncan's multiple range test (SAS Institute Inc., Cary, NC). Effects were considered significant when $P < 0.05$.

RESULTS

Experiment 1

No influence ($P > 0.05$) was observed on laying hen performance and egg quality parameters (data not shown). Mean values for the following parameters were recorded: hen-day egg production (76.2%), egg weight (62.2 g), feed intake (115 g/d), feed conversion rate (2.45 feed/egg, g/g), eggshell hardness (42.11 N/cm^2), Haugh unit (89.62), Roche fan color (7.1), shell thickness (0.29 mm), and albumen height (8.14 mm).

The effects of dietary vitamin E supplementation on liver, egg yolk, and plasma α-tocopherol and retinol in the 4th week and 8th week are shown in Figure 1 and Figure 2. In the 4th week and 8th week, adding different levels of vitamin E to the diets, the contents of α-tocopherol and retinol were significantly increased ($P < 0.05$) in liver. In the 4th week, a significant effect of vitamin E supplementation was observed, as hens fed with the higher concentration of vitamin E had higher egg yolk α-tocopherol ($P < 0.05$). In the 4th week, the content of retinol in egg yolk significantly increased ($P < 0.05$), when dietary vitamin E supplementation reached 80 IU/kg. In the 8th week, the content of retinol in egg yolk also significantly increased ($P < 0.05$), when dietary vitamin E supplementation amounted to 20 and 80 IU/kg. In the 8th week, however, higher vitamin E (320, 1,280 IU/kg) supplementations significantly decreased ($P < 0.05$) vitamin A concentrations of egg yolk. The plasma concentration of α-tocopherol showed a maximum at a supplementation level of 1,280 IU/kg of diet, and the plasma concentration of retinol was independent of the vitamin E supply.

The content of RBP in liver and plasma at different levels of dietary vitamin E are shown in Table 3. In the 4th week, the content of RBP in liver decreased with the increasing dietary vitamin E concentrations. When the dietary vitamin E was 1,280 IU/kg, it significantly reduced the liver RBP ($P < 0.05$), and compared with the control group, the content of liver RBP decreased by 28.1%. In the 8th week, the content of RBP in the 320 and 1,280 IU/kg dietary vitamin E groups was significantly ($P < 0.05$) decreased compared with the control group. Supplementation with vitamin E in either group had no significant effect on the plasma RBP concentration throughout the experimental period.

The relative liver weight, content of microsomal protein, and CYP450 are presented in Table 4. No differences were observed in the 4th week and 8th week.

The mRNA expression of *RBP* and *CYP26A1* involved in vitamin A metabolism was measured by semi-

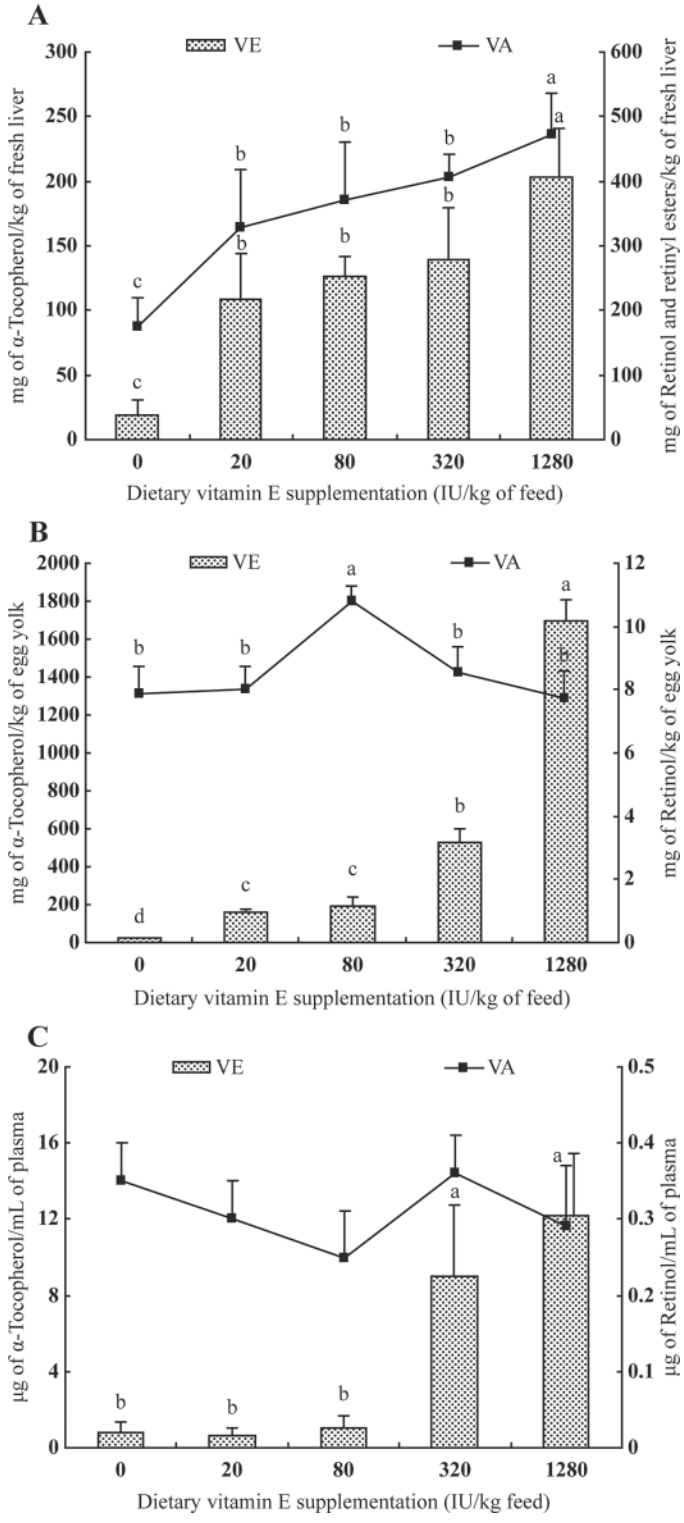

Figure 1. Effects of dietary vitamin E supplementation (IU/kg of feed) on α-tocopherol (VE) and retinol (VA) concentration in (A) fresh liver (mg/kg), (B) egg yolk (mg/kg), and (C) plasma (μg/mL) in the 4th week. n = 6; error bars represent SE. [a–d]Means at the same week followed by different letters differ significantly ($P < 0.05$).

quantitative reverse-transcription PCR with cDNA from liver of laying hens (Figure 3). A marked down-regulation of *RBP* mRNA expression was observed in liver from the 1,280 IU of vitamin E/kg of supplementation group. The *CYP26A1* mRNA expression was not affected by different vitamin E treatments.

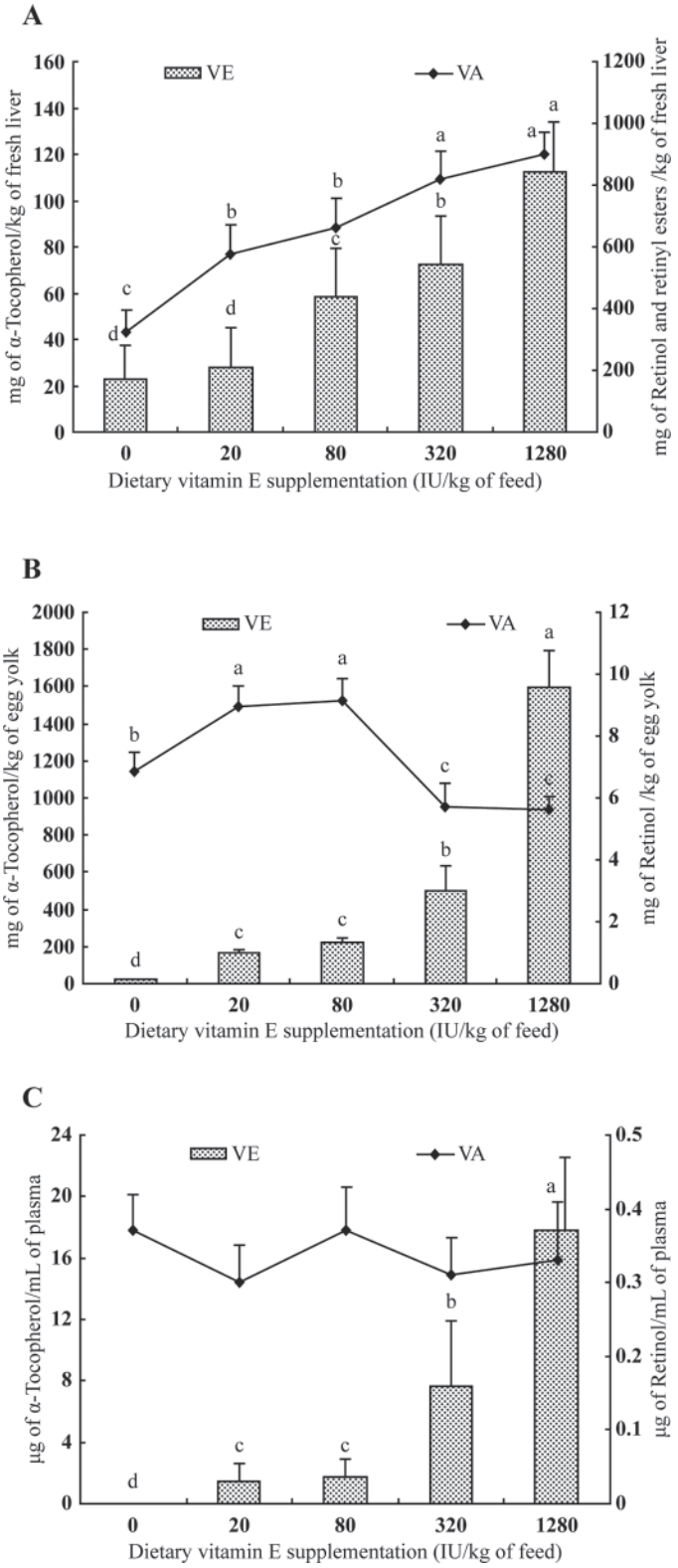

Figure 2. Effects of dietary vitamin E supplementation (IU/kg of feed) on α-tocopherol (VE) and retinol (VA) concentration in (A) fresh liver (mg/kg), (B) egg yolk (mg/kg), and (C) plasma (μg/mL) in the 8th week. n = 6; error bars represent SE. [a–d]Means at the same week followed by different letters differ significantly ($P < 0.05$).

Experiment 2

Four levels of α-tocopherol were tested by reacting with hepatocytes from α-tocopherol-depleted laying

Table 3. The effect of supplementing different levels of vitamin E in diets on retinol binding protein (RBP) concentration in liver and plasma from laying hens

	Vitamin E supplemental level[1] (IU/kg)						
Item	0	20	80	320	1,280	SEM	*P*-value
Liver RBP (nmol/g)							
Wk 4	1.14[a]	1.09[a]	1.07[a]	0.97[ab]	0.89[b]	0.03	0.044
Wk 8	1.56[a]	1.49[ab]	1.48[ab]	1.37[b]	1.35[b]	0.02	0.023
Plasma RBP (μmol/L)							
Wk 4	1.11	0.90	1.12	0.95	0.93	0.04	0.239
Wk 8	1.11	1.15	1.08	1.15	1.06	0.02	0.590

[a,b]Means in row without a common superscript differ significantly, $P < 0.05$.

[1]Results are shown as means ± SEM, and means represent 6 replicates per treatment.

hens. The LDH activity, total protein, and RBP content were measured in the culture medium and cells after 20 min (Table 5). There were no differences observed in LDH activity and total protein concentration. However, when culture medium contained 100 μ*M* α-tocopherol, RBP secretion was significantly inhibited ($P < 0.05$), approximately 26.9% compared with the control group.

The PCR products representing *RBP* and *CYP26A1* from hepatocytes are shown in Figure 4. A marked downregulation of *RBP* mRNA expression was observed in hepatocytes in 100 μ*M* α-tocopherol groups, whereas *CYP26A1* mRNA expression was not affected by different α-tocopherol treatments.

DISCUSSION

Consistent with our results, several studies have not found any positive (Kling and Soares, 1980; Grobas et al., 2002) or negative (Meluzzi et al., 2000; Galobart et al., 2001) effects on the performance and egg quality of laying hens when vitamin E was added at the same level (i.e., 1,280 mg/kg).

In our study, diets containing different vitamin E concentrations were fed to laying hens. As expected, the α-tocopherol and retinol concentrations in liver were distinctly higher after feeding excessive vitamin E. The retinol concentration of egg yolk was significantly decreased from 6.87 to 5.62 mg/kg in the 8th week, whereas α-tocopherol in the egg increased without reaching a plateau. Plasma retinol was not affected by dietary or plasma vitamin E concentrations. Similar observations were made by other groups (Sklan, 1983; Sünder et al., 1999; Sünder and Flachowsky, 2001).

In the present study, the content of *RBP* and its mRNA expression in liver, plasma, and hepatocytes from laying hens were measured to determine whether vitamin E affected vitamin A, caused by RBP. The findings indicate that the concentration of *RBP* in plasma is not affected by excess vitamin E in the diet, but a marked decrease in liver and hepatocyte *RBP* synthesis would be expected to result in a higher concentration of vitamin A in liver because vitamin A cannot be excreted from the liver without RBP (Noy, 2000). The observation that plasma *RBP* content was not affected may be due to the fact that extrahepatic tissues also can secrete *RBP* (Soprano et al., 1986; Whitman et al., 1990), thus maintaining the RBP stability in circulatory system. Other studies have also shown that RBP can be regulated by fat-soluble vitamins. The *RBP* secretion from rat liver was specifically inhibited during retinol deficiency; however, when resupplying the deficient rats with retinol rapidly, its release would be specifically stimulated (Muto et al., 1972; Goodman, 1984). Muto et al. (1972) reported that plasma RBP declined from 50 ± 4 μg/mL to 13 ± 2 μg/mL in vitamin A-deficient rats during 75 d, and a very rapid increase in RBP level from 14 to 56 μg/mL was observed in 5 h when 24 μg of vitamin A was administrated orally to deficient rats on d 53. In contrast, addition of exogenous retinol did not appear to influence RBP secretion in normal rat hepatocytes (Dixon and Goodman, 1987). In our study, both in vivo and in vitro experiments have

Table 4. The effect of supplementing different levels of vitamin E on hepatic microsomal cytochrome P450 (CYP450) in laying hens

	Vitamin E supplemental level[1] (IU/kg)						
Item	0	20	80	320	1,280	SEM	*P*-value
Relative liver weight[2] (%)							
Wk 4	1.18	1.08	1.58	1.04	1.48	0.08	0.155
Wk 8	2.08	1.69	1.74	1.70	1.82	0.07	0.348
Microsomal protein (mg/g of liver wet weight)							
Wk 4	3.63	2.78	3.02	3.92	4.35	0.23	0.177
Wk 8	4.43	5.14	5.25	5.02	5.20	0.24	0.831
CYP450 (nmol/mg of protein)							
Wk 4	1.46	1.42	1.52	1.45	1.52	0.03	0.595
Wk 8	2.14	2.07	2.20	2.30	2.09	0.08	0.896

[1]Results are shown as means ± SEM, and means represent 6 replicates per treatment.

[2]Relative liver weight (%) = (liver weight/100 g of BW) × 100.

Table 5. Effects of α-tocopherol in different concentrations on lactate dehydrogenase activity (LDH), total protein, and retinol binding protein (RBP) content in hepatocytes from laying hens

	α-Tocopherol concentration[1] (μ*M*)					
Item	0	10	50	100	SEM	*P*-value
LDH activity (U/L)	253	235	214	203	20	0.853
Total protein (mg/mL)	0.13	0.14	0.11	0.12	0.01	0.643
RBP (nmol/L)	1.24[a]	1.14[a]	1.05[ab]	0.90[b]	0.04	0.020

[a,b]Means in row without a common superscript differ significantly, $P < 0.05$

[1]Results are shown as means ± SEM, and means represent 4 replicates per treatment.

shown that excessive vitamin E (1,280 IU/kg of DL-α-tocopheryl acetate supplementation in diet and 100 μ*M* α-tocopherol in cell medium) inhibit the synthesis of *RBP* in liver and hepatocytes. The observations that the activity of LDH and the content of total protein are not affected implies that RBP is specifically decreased by vitamin E because no significant change of LDH activity and total protein content indicates that all cells are in the similar and comparable conditions.

Although semiquantitative reverse-transcription PCR is not the most accurate method, it is useful in determining qualitative and quantitative gene expression (Meadus, 2003; Kuo and Chang, 2007). We use this method to verify the effects of vitamin E on *RBP* and *CYP26A1* mRNA expression according to the literature (Morris and Davila, 1996; Meadus, 2003). Our results first indicate that the *RBP* mRNA expression in liver and hepatocytes is downregulated by 1,280 IU/kg of vitamin E supplication and 100 μ*M* α-tocopherol, respectively. Therefore, we conclude that *RBP* mRNA expression is inhibited by excessive vitamin E supplementation.

Megadoses of vitamin E do not lower the contents of hepatic microsomal protein, CYP450, and *CYP26A1* mRNA expression in liver and hepatocytes from laying hens. In contrast, *CYP26A1* expression in normal human neonatal foreskin keratinocytes is extremely sensitive to both exogenous retinol acid and increased endogenous retinol acid due to CYP26 inhibition (Pavez-Loriè et al., 2009). Mustacich et al. (2009) demonstrated that increased hepatic α-tocopherol modulates its own concentrations through increasing the xenobiotic metabolism. They fed mice with a 1,000 IU vitamin E supplementation diet for 4 mo; in this group, catabolic gene expression of *P450* oxidoreductase and *CYP3A11* increased 1.6- and 4-fold, respectively, com-

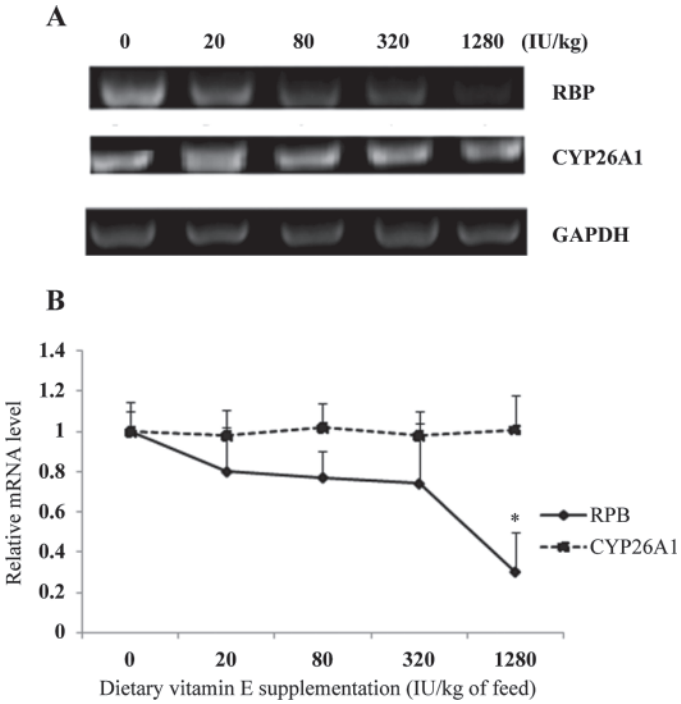

Figure 3. Effects of dietary vitamin E supplementation (IU/kg of feed) on retinol binding protein (*RBP*) and cytochrome P450 family 26 subfamily A polypeptide 1 (*CYP26A1*) mRNA expression in liver (n = 3). (A) The gel electrophoresis of *RBP*, *CYP26A1*, and *GAPDH* PCR amplification products. (B) Densitometric evaluation of relative levels of *RBP* and *CYP26A1* mRNA normalized by the *GAPDH* mRNA level in each sample. Error bars represent SE. Significance is represented as (*), $P < 0.05$.

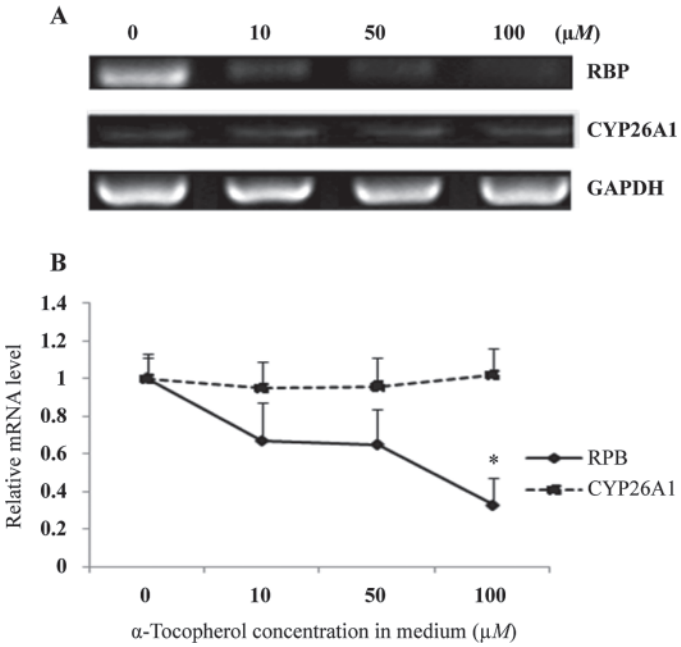

Figure 4. Effects of α-tocopherol in medium on retinol binding protein (*RBP*) and cytochrome P450 family 26 subfamily A polypeptide 1 (*CYP26A1*) mRNA expression in hepatocytes (n = 3). (A) The gel electrophoresis of *RBP*, *CYP26A1*, and *GAPDH* PCR amplification products. (B) Densitometric evaluation of relative levels of *RBP* and *CYP26A1* mRNA normalized by the *GAPDH* mRNA level in each sample. Error bars represent SE. Significance is represented as (*), $P < 0.05$.

pared with the control group. Thus, we suggest that vitamin E may not influence the catabolism of vitamin A.

In summary, our study indicates that vitamin E increases the level of vitamin A in liver of laying hens. Particularly, we demonstrate that vitamin E in 320 and 1,280 IU/kg of diet or 100 μM medium inhibits RBP synthesis, but does not affect *CYP26A1* mRNA expression in liver and hepatocytes.

ACKNOWLEDGMENTS

The work has been financially supported by an earmarked fund for the National System for Layer Production Technology CARS-41-K16. We thank the following colleagues for their assistance: Qingsheng Liu and Zhiying Fan of the Feed Research Institute of the Chinese Academy of Agricultural Sciences (Beijing, China) for their suggestions; and Jianzhong Shen of the College of Veterinary Medicine of China Agricultural University for hepatic microsome preparation.

REFERENCES

Dixon, J. L., and D. S. Goodman. 1987. Studies on the metabolism of retinol-binding protein by primary hepatocytes from retinol-deficient rats. J. Cell. Physiol. 130:14–20.

Fraslin, J. M., L. Touquette, M. Douaire, Y. Menezo, J. C. Guillemot, and J. Mallard. 1992. Isolation and long-term maintenance of differentiated adult chicken hepatocytes in primary culture. In Vitro Cell. Dev. Biol. 28A:615–620.

Galobart, J., A. C. Barroeta, M. D. Baucells, L. Cortinas, and F. Guardiola. 2001. α-tocopherol transfer efficiency and lipid oxidation in fresh and spray-dried eggs enriched with ω3-polyunsaturated fatty acids. Poult. Sci. 80:1496–1505.

Goodman, D. S. 1984. Overview of current knowledge of metabolism of vitamin A and carotenoids. J. Natl. Cancer Inst. 73:1375–1379.

Grobas, S., J. Mendez, B. C. Lopez, B. C. De, and G. G. Mateos. 2002. Effect of vitamin E and A supplementation on egg yolk α-tocopherol concentration. Poult. Sci. 81:376–381.

Hao, C., W. Liu, X. Luan, Y. Li, H. Gui, Y. Peng, J. Shen, G. Hu, and J. Yang. 2010. Aquaporin-4 knockout enhances astrocyte toxicity induced by 1-methyl-4-phenylpyridinium ion and lipopolysaccharide via increasing the expression of cytochrome P4502E1. Toxicol. Lett. 198:225–231.

Kling, L. J., and J. H. Soares Jr. 1980. Vitamin E deficiency in the Japanese quail. Poult. Sci. 59:2352–2354.

Kuo, Y. Y., and Z. F. Chang. 2007. GATA-1 and Gfi-1B interplay to regulate Bcl-xL transcription. Mol. Cell. Biol. 27:4261–4272.

Lampen, A., S. Meyer, and H. Nau. 2001. Effects of receptor-selective retinoids on *CYP26* gene expression and metabolism of all-*trans*-retinoic acid in intestinal cells. Drug Metab. Dispos. 29:742–747.

Liu, M. J., Z. Wang, H. X. Li, R. C. Wu, Y. Z. Liu, and Q. Y. Wu. 2004. Mitochondrial dysfunction as an early event in the process of apoptosis induced by woodfordinIin human leukemia K562 cells. Toxicol. Appl. Pharmacol. 194:141–155.

Long, K. Z., J. I. Santos, J. L. Rosado, T. Estrada-Garcia, M. Haas, A. A. I. Mamun, H. L. DuPont, and N. N. Nanthakumar. 2011. Vitamin A supplementation modifies the association between mucosal innate and adaptive immune responses and resolution of enteric pathogen infections. Am. J. Clin. Nutr. 93:578–585.

Mao, J., S. Chen, Z. Na, Y. Xhang, Y. Huang, and Y. Li. 1996. Frozen storage of urine samples before ELISA measurement of retinol-binding protein. Clin. Chem. 42:466–467.

Meadus, W. J. 2003. A semi-quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on porcine muscle PPAR gene expression. Biol. Proced. Online 5:20–28.

Meluzzi, A., F. Sirri, G. Manfreda, N. Tallarico, and A. Franchini. 2000. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. Poult. Sci. 79:539–545.

Morris, D. L., and J. C. Davila. 1996. Analysis of rat cytochrome P450 isoenzyme expression using semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). Biochem. Pharmacol. 52:781–792.

Mustacich, D. J., K. Gohil, R. S. Bruno, M. Yan, S. W. Leonard, E. Ho, C. E. Cross, and M. G. Traber. 2009. Alpha-tocopherol modulates genes involved in hepatic xenobiotic pathways in mice. J. Nutr. Biochem. 20:469–476.

Muto, Y., J. E. Smith, P. O. Milch, and D. S. Goodman. 1972. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J. Biol. Chem. 247:2542–2550.

Napoli, J. L., A. M. McCormick, B. O'Meara, and E. A. Dratz. 1984. Vitamin A metabolism: α-Tocopherol modulates tissue retinol levels in vivo, and retinyl palmitate hydrolysis in vitro. Arch. Biochem. Biophys. 230:194–202.

Noy, N. 2000. Retinoid-binding proteins: Mediators of retinoid action. Biochem. J. 348:481–495.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. National Academy Press, Washington, DC.

Pavez-Loriè, E., J. C. Chamcheu, A. Vahlquist, and H. Torma. 2009. Both all-*trans*-retinoic acid and cytochrome P450 (CYP26) inhibitors affect the expression of vitamin A metabolizing enzymes and retinoid biomarkers in organotypic epidermis. Arch. Dermatol. Res. 301:475–485.

Perlmann, T. 2002. Retinoid metabolism: A balancing act. Nat. Genet. 31:7–8.

Ray, W. J., G. Bain, M. Yao, and D. I. Gottlieb. 1997. CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family. J. Biol. Chem. 272:18702–18708.

Rifkind, A. B., M. Troeger, and H. Muschick. 1982. Kinetic evidence for heterogeneous responsiveness of mixed function oxidase isozymes to inhibition and induction by allylisopropylacetamide in chick embryo liver. J. Biol. Chem. 257:11717–11727.

Sato, K., Y. Cho, S. Tachibana, T. Chiba, W. J. Schneider, and Y. Akiba. 2005. Impairment of VLDL secretion by medium-chain fatty acids in chicken primary hepatocytes is affected by the chain length. J. Nutr. 135:1636–1641.

Sheridan, P. A., and M. A. Beck. 2008. The immune response to herpes simplex virus encephalitis in mice is modulated by dietary vitamin E. J. Nutr. 138:130–137.

Sklan, D. 1983. Vitamin A absorption and metabolism in the chick: Response to high dietary intake and to tocopherol. Br. J. Nutr. 50:401–407.

Soprano, D. R., K. J. Soprano, and D. S. Goodman. 1986. Retinol-binding protein messenger RNA levels in liver and in extrahepatic tissues of the rat. J. Lipid Res. 27:166–171.

Sünder, A., and G. Flachowsky. 2001. Influence of high vitamin E dosages on retinol and carotinoid concentration in body tissues and eggs of laying hens. Arch. Tierernähr. 55:43–52.

Sünder, A., I. Halle, and G. Flachowsky. 1999. Vitamin E hypervitaminosis in laying hens. Arch. Tierernährg. 52:185–194.

Swindell, E. C., C. Thaller, S. Sockanathan, M. Petkovich, T. M. Jessell, and G. Eichele. 1999. Complementary domains of retinoic acid production and degradation in the early chick embryo. Dev. Biol. 216:282–296.

White, J. A., B. Beckett-Jones, Y. D. Guo, F. J. Dilworth, J. Bonasoro, G. Jones, and M. Pektovich. 1997. cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450. J. Biol. Chem. 272:18538–18541.

Whitman, M. M., D. C. Harnish, K. J. Soprano, and D. R. Soprano. 1990. Retinol-binding protein mRNA is induced by estrogen in the kidney but not in the liver. J. Lipid Res. 31:1483–1490.

Yang, H., and S. Jiang. 2006. Methodological study on determination of activities of hepatic microsomalcytochrome P450s in chickens. Vet. Sci. China 36:147–150. (In Chinese)

Zanotti, G., V. Calderone, M. Beda, G. Malpeli, C. Folli, and R. Berni. 2001. Structure of chicken plasma retinol-binding protein. Biochim. Biophys. Acta 1550:64–69.