Effects of Vitamin E on Immune Function, Tissue α-Tocopherol Deposition, and Lipoprotein Lipase and Fatty Acid-Binding Protein Gene Expression in Sanhuang Broiler Chickens: Postprint

Authors: Liu Minyan, Bu Zeming, Li Yuanyuan, Liu Wentao, Su Lisui, Liang Mingzhen

Date: 2018-12-20T00:00:00+00:00

Abstract

The present experiment aimed to investigate the effects of different dietary vitamin E levels on immune function, tissue α-tocopherol deposition, and the expression of lipoprotein lipase (LPL) and fatty acid-binding protein genes in Sanhuang broiler chickens. A total of 256 80-day-old Guangxi Sanhuang broiler chickens were randomly divided into 4 groups, with each group receiving 0 (control), 50, 100, or 150 mg/kg vitamin E supplementation to the basal diet, respectively. Each group consisted of 4 replicates with 16 chickens per replicate. The preliminary period lasted 5 days, and the formal experimental period lasted 35 days. The results showed that, compared with the control group: 1) Dietary supplementation with 50 mg/kg vitamin E significantly increased serum immunoglobulin A content ($P<0.05$); dietary supplementation with 150 mg/kg vitamin E significantly increased serum immunoglobulin M content ($P<0.05$); dietary supplementation with different levels of vitamin E had no significant effects on spleen index and serum contents of interleukin-2, tumor necrosis factor-α, interferon-γ, and immunoglobulin G ($P>0.05$). 2) Dietary supplementation with 50, 100, and 150 mg/kg vitamin E all significantly increased liver α-tocopherol content ($P<0.05$); dietary supplementation with 150 mg/kg vitamin E significantly increased breast muscle α-tocopherol content ($P<0.05$); dietary supplementation with different levels of vitamin E had no significant effect on leg muscle α-tocopherol content ($P>0.05$). 3) Dietary supplementation with 100 and 150 mg/kg vitamin E both significantly increased liver LPL gene expression ($P<0.05$); dietary supplementation with 150 mg/kg vitamin E significantly increased liver heart-type fatty acid-binding protein (H-FABP) gene expression ($P<0.05$); dietary supplementation with 50, 100, and 150 mg/kg vitamin E all significantly increased liver liver-type fatty acid-binding protein (L-

FABP) gene expression (P<0.05); dietary supplementation with different levels of vitamin E had no significant effect on liver adipocyte-type fatty acid-binding protein (A-FABP) gene expression (P>0.05). 4) Dietary vitamin E supplementation level showed a significant positive correlation with liver LPL, H-FABP, and L-FABP gene expression in Guangxi Sanhuang broiler chickens (P<0.05). In conclusion, dietary supplementation with high levels (150 mg/kg) of vitamin E can improve immune function, increase tissue α-tocopherol deposition, and regulate liver LPL, H-FABP, and L-FABP gene expression, thereby affecting lipid metabolism in Guangxi Sanhuang broiler chickens.

Full Text

Effects of Vitamin E on Immune Function, α-Tocopherol Deposition, and Gene Expressions of Lipoprotein Lipase and Fatty Acid-Binding Proteins in Guangxi Sanhuang Broiler Chickens

LIU Minyan[1], BU Zeming[2], LI Yuanyuan[1], LIU Wentao[1], SU Lisui[3], LIANG Mingzhen[1]*

[1]College of Animal Science and Technology, Guangxi University, Nanning 530004, China
[2]Institute of Veterinary Drug Control of Guangxi Zhuang Autonomous Region, Nanning 530001, China
[3]Guangxi Fufeng Group, Nanning 530001, China

Abstract: This study investigated the effects of different dietary vitamin E levels on immune function, α-tocopherol deposition, and gene expressions of lipoprotein lipase (LPL) and fatty acid-binding proteins in Guangxi Sanhuang broiler chickens. A total of 256 healthy 80-day-old Guangxi Sanhuang chickens were randomly allocated into four groups with four replicates per group and 16 chickens per replicate. The basal corn-soybean diet was supplemented with 0 (control), 50, 100, or 150 mg/kg vitamin E. The pre-experimental period lasted 5 days, followed by a 35-day formal experimental period. The results showed that compared with the control group: (1) dietary supplementation with 50 mg/kg vitamin E significantly increased serum immunoglobulin A (IgA) content (P<0.05), while 150 mg/kg vitamin E significantly increased serum immunoglobulin M (IgM) content (P<0.05); however, different vitamin E levels had no significant effects on spleen index or serum contents of interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), interferon-γ, or immunoglobulin G (IgG) (P>0.05). (2) Supplementation with 50, 100, and 150 mg/kg vitamin E all significantly increased hepatic α-tocopherol content (P<0.05), and 150 mg/kg vitamin E significantly increased breast muscle α-tocopherol content (P<0.05), though different vitamin E levels showed no significant effect on thigh muscle α-tocopherol content (P>0.05). (3) Dietary vitamin E at 100 and 150 mg/kg significantly upregulated hepatic LPL gene expression (P<0.05); 150 mg/kg

vitamin E significantly increased hepatic heart-type fatty acid-binding protein (H-FABP) gene expression ($P<0.05$); and 50, 100, and 150 mg/kg vitamin E all significantly enhanced hepatic liver-type fatty acid-binding protein (L-FABP) gene expression ($P<0.05$), while adipocyte-type fatty acid-binding protein (A-FABP) gene expression remained unaffected ($P>0.05$). (4) Significant positive correlations were observed between dietary vitamin E level and hepatic expression of LPL, H-FABP, and L-FABP genes ($P<0.05$). In conclusion, high-level dietary vitamin E supplementation (150 mg/kg) can improve immune function, enhance α-tocopherol deposition in tissues, and modulate hepatic expression of LPL, H-FABP, and L-FABP genes, thereby influencing lipid metabolism in Guangxi Sanhuang broiler chickens.

Keywords: vitamin E; Guangxi Sanhuang chicken; immune function; α-tocopherol deposition; gene expression level

Introduction

When animals are exposed to adverse environmental stimuli, their physiological status changes, triggering a series of neuroendocrine and immune responses that can compromise immunity and resistance. These alterations ultimately affect growth, production performance, and may even cause mortality under severe conditions. Enhancing the immune function of livestock and poultry is therefore crucial for healthy development and reduced mortality under inevitable environmental stress. Vitamin E serves as a versatile immunomodulator that regulates normal tissue function in most animal organs, and appropriate dietary supplementation has been shown to improve immune function in poultry. As the most biologically active form of vitamin E, α-tocopherol represents the primary storage form, making it important to investigate whether its tissue deposition increases with higher dietary vitamin E intake.

The liver is the primary site of lipid metabolism in chickens, a process that depends on various regulatory factors including lipoprotein lipase (LPL) and fatty acid-binding proteins (FABPs). LPL plays a crucial role in hepatic lipid metabolism by hydrolyzing triglycerides in very low-density lipoproteins (VLDL) to produce glycerol and free fatty acids. FABPs transport long-chain fatty acids and regulate intracellular fatty acid concentrations, thereby modulating fatty acid metabolism. Among the nine FABP types identified based on tissue distribution, this study focused on three closely associated with hepatic lipid metabolism: adipocyte-type FABP (A-FABP), heart-type FABP (H-FABP), and liver-type FABP (L-FABP). Due to its lipophilic and antioxidant properties, vitamin E was recognized in the 1980s as having signal transduction and gene regulatory functions. This experiment examined how different dietary vitamin E levels affect immune function and α-tocopherol deposition in Guangxi Sanhuang broiler chickens, with particular emphasis on investigating whether vitamin E can regulate lipid metabolism-related candidate genes, providing evi-

dence for whether vitamin E can improve lipid metabolism through modulation of gene expression.

Materials and Methods

Experimental Animals and Design A single-factor completely randomized design was employed using 256 healthy 80-day-old Guangxi Sanhuang hens with similar body weights. The chickens were randomly divided into four groups, each consisting of four replicates with 16 birds per replicate. A corn-soybean basal diet was formulated, with the control group receiving no supplementation and experimental groups I, II, and III receiving 50, 100, and 150 mg/kg vitamin E, respectively. The study included a 5-day pre-experimental period followed by a 35-day formal experimental period. All birds were housed in cages and fed powdered feed ad libitum with free access to water under natural lighting and ventilation. The vitamin E supplement used was DL-α-tocopheryl acetate with 50% active ingredient content.

Experimental Diets The composition and nutrient levels of the basal diet are presented in . The premix provided per kilogram included: vitamin A 70,000–250,000 IU, vitamin B_1 \$ $30mg, vitaminB\{2\}\$$ \$ $80mg, vitaminB\{6\}\$$ \$ $70mg, vitaminB\{12\}\$$ \$ $0.15mg, vitaminD\{3\}\$$ 42,000–120,000 IU, vitamin K_3 45–125 mg, pantothenic acid \$ \$350 mg, nicotinic acid \$ \$800 mg, biotin \$ \$7.0 mg, choline \$ \$8.0 g, folic acid \$ \$15 mg, Mn 1,000–3,700 mg, Zn 1,250–3,700 mg, Fe 1,000–12,000 mg, Cu 210–800 mg, I 4–125 mg, Se 4.0–12.0 mg, Co 4–50 mg, TP 20–50 g, and NaCl 20–50 g. Crude protein, calcium, and available phosphorus contents were measured values, while other nutrient levels were calculated values.

Management Practices All experimental chickens were housed in the same facility under cage-rearing conditions. Birds received powdered feed ad libitum and free access to water with natural lighting and ventilation. Management and immunization procedures followed conventional farm practices.

Sample Collection At the conclusion of the 120-day rearing period, two chickens were randomly selected from each replicate (eight per group) for sampling. Prior to slaughter, feed was withheld for 12 hours while water remained available. During processing, the liver and left breast and thigh muscles were carefully dissected, rinsed with double-distilled water to remove blood and debris, weighed, and sampled. Tissue samples were snap-frozen in liquid nitrogen and subsequently stored at -80°C for determination of α-tocopherol content and gene expression analysis.

Laboratory Analyses Immune Indices: Serum IL-2, TNF-α, and interferon-γ contents were measured by enzyme-linked immunosorbent assay (ELISA), while IgG, IgA, and IgM levels were determined using an automatic

biochemical analyzer (ACA). The spleen index was calculated as the percentage of spleen weight relative to body weight.

α-Tocopherol Content: Hepatic, breast muscle, and thigh muscle α-tocopherol contents were determined by high-performance liquid chromatography according to the national standard method (GB/T 9695.30–2008).

Gene Expression Analysis: Total RNA was extracted using the Trizol method following the protocol of GenStar' s RNA extraction kit, with concentrations and purity assessed by spectrophotometry. Complementary DNA (cDNA) was synthesized from total RNA using a cDNA synthesis kit according to manufacturer instructions. Specific primers were designed based on chicken sequences from GenBank for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), LPL, A-FABP, H-FABP, and L-FABP genes. Real-time quantitative PCR (RT-PCR) was performed in a 20 L reaction volume containing 1 L cDNA, 1 L each of forward and reverse primers, 10 L 2×RealStar Green Fast Mixture with ROX II, and 7 L RNase-free water. The thermal cycling program consisted of initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 0.5 min, 56°C for 0.5 min, and 72°C for 0.5 min, with a final extension at 72°C for 7 min. Gene expression levels were quantified using the 2^(-ΔΔCt) method.

Statistical Analysis All data were analyzed by one-way ANOVA using SPSS 19.0 software, followed by Duncan' s multiple comparison test. Results are expressed as means ± standard deviation. Significance was declared at $P<0.05$.

Results

Effects of Vitamin E on Serum Immune Indices and Immune Organ Index As shown in , dietary supplementation with 50 mg/kg vitamin E significantly increased serum IgA content compared with the control group ($P<0.05$), while 150 mg/kg vitamin E significantly elevated serum IgM content ($P<0.05$). However, different vitamin E supplementation levels had no significant effects on serum IL-2, TNF-α, interferon-γ, or IgG contents, nor on spleen index ($P>0.05$).

Effects of Vitamin E on α-Tocopherol Deposition in Tissues presents the effects of vitamin E on tissue α-tocopherol deposition. Supplementation with 50, 100, and 150 mg/kg vitamin E all significantly increased hepatic α-tocopherol content ($P<0.05$). Additionally, 150 mg/kg vitamin E significantly increased breast muscle α-tocopherol content ($P<0.05$). Although dietary vitamin E levels showed no significant effect on thigh muscle α-tocopherol content ($P>0.05$), a tendency for increased deposition was observed with higher supplementation levels.

Effects of Vitamin E on Hepatic LPL and FABPs Gene Expression As illustrated in , hepatic expression of LPL, H-FABP, and L-FABP genes increased with higher dietary vitamin E levels. Compared with the control group,

100 and 150 mg/kg vitamin E significantly upregulated hepatic LPL gene expression ($P<0.05$). Supplementation with 150 mg/kg vitamin E significantly increased hepatic H-FABP gene expression ($P<0.05$), while 50, 100, and 150 mg/kg vitamin E all significantly enhanced hepatic L-FABP gene expression ($P<0.05$). Dietary vitamin E levels had no significant effect on hepatic A-FABP gene expression ($P>0.05$).

Correlation Between Dietary Vitamin E Level and Tissue Parameters demonstrates that dietary vitamin E level was significantly positively correlated with thigh muscle α-tocopherol content and hepatic expression of LPL, H-FABP, and L-FABP genes in Guangxi Sanhuang chickens ($P<0.05$).

Discussion

Effects of Vitamin E on Immune Function in Guangxi Sanhuang Chickens Previous research has shown that dietary vitamin E supplementation significantly increases serum IgA content in ducklings, with elevated trends observed for IgG and IgM. International studies also indicate that in ovo vitamin E injection improves hatchability and significantly increases serum IgM and IgA contents at 42 days of age, with IgG content also showing improvement. In the current study, serum IgG, IgA, and IgM contents increased to varying degrees across all treatment groups compared with the control, while serum IL-2 content showed an upward trend. These findings suggest that appropriate dietary vitamin E levels can enhance immunoglobulin synthesis and promote IL-2 secretion, thereby strengthening immunity and resistance to adverse environmental conditions. Jiang et al. reported that vitamin E deficiency suppresses immune organ development, while vitamin E supplementation significantly reduces serum TNF-α content. The present results partially support this conclusion, indicating that vitamin E inhibits TNF-α secretion, alleviates inflammatory responses, and promotes normal immune system function. Overall, increasing dietary vitamin E levels in Guangxi Sanhuang chickens appears beneficial for the development of immune response factors and consequently improves immune function.

Effects of Vitamin E on Tissue α-Tocopherol Content α-Tocopherol represents the most biologically active and abundant isoform of vitamin E, making its tissue content a reliable indicator of vitamin E deposition status. Zhang et al. demonstrated that increasing dietary vitamin E levels significantly elevates hepatic α-tocopherol content in laying hens, while other studies have shown that α-tocopheryl acetate supplementation significantly increases serum α-tocopheryl acetate levels in broilers. Earlier research also reported that higher dietary vitamin E levels significantly increased α-tocopherol content in egg yolk, liver, and muscle of laying hens. The current findings align with these studies, confirming a strong positive correlation between dietary vitamin E intake and tissue deposition. Notably, hepatic α-tocopherol deposition was several-fold to

ten-fold higher than that in breast or thigh muscle, suggesting that the liver may serve as a critical regulatory site for vitamin E function in the body.

Effects of Vitamin E on LPL and FABPs Gene Expression Hepatic LPL gene expression is closely associated with lipid metabolism, showing positive relationships with intramuscular fat deposition and serving as a key factor influencing plasma lipid levels. This study explored whether vitamin E could positively modulate LPL expression. The results indicate that high-level vitamin E supplementation significantly increased hepatic LPL gene expression, with a significant positive correlation between vitamin E level and LPL expression, suggesting that vitamin E may influence lipid metabolism indirectly through modulation of LPL gene expression. A-FABP, released by adipocytes and macrophages, participates in intercellular lipid transport and serves as a metabolic and vascular risk biomarker. In this experiment, vitamin E exerted only non-significant fine-tuning effects on hepatic A-FABP gene expression, indicating it does not adversely affect lipid metabolism through excessive upregulation. H-FABP is closely related to adipose tissue development and function, considered a candidate gene for lipid metabolism that influences fat deposition. The present study found that high-level vitamin E significantly increased hepatic H-FABP gene expression with a significant positive correlation, suggesting vitamin E may regulate tissue fat deposition through modulation of H-FABP expression. Hepatic L-FABP gene expression is influenced by various factors, including high-fat diets that increase its expression in rats, and previous research has shown that increasing dietary vitamin E levels significantly elevates hepatic L-FABP expression in mule ducks. The current results are consistent with these findings. Collectively, these results demonstrate that vitamin E can influence the expression of lipid metabolism-related genes including LPL, H-FABP, and L-FABP, providing evidence that vitamin E may affect lipid metabolism through modulation of gene expression, though the specific mechanisms require further investigation.

Conclusion

Dietary supplementation with high-level vitamin E (150 mg/kg) can improve immune function, enhance α-tocopherol deposition in tissues, and modulate hepatic expression of LPL, H-FABP, and L-FABP genes, thereby influencing lipid metabolism in Guangxi Sanhuang broiler chickens.

References

[1] YU Liangbin. Causes and countermeasures for decreased immunity in large-scale pig farms[J]. China Animal Husbandry and Veterinary Medicine, 2011, 8(11): 67–69.
[2] SUN Yongfeng, LI Liyong, WU Mingyu. Research progress on vitamin E nutrition[J]. Modern Agricultural Science and Technology, 2007(23): 209–210.
[3] WU Yuanyuan, WANG Yuxiang, LI Hui. Research progress on lipid

metabolism-related factors in chicken liver[J]. Animal Husbandry and Veterinary Medicine, 2013, 45(1): 91-95.
[4] LIU Shunde, LI Na, GAO Xiaoyan, et al. Research progress on association between animal FABP family genes and fat deposition[J]. Journal of Agricultural Sciences, 2010, 31(3): 44-48.
[5] GALLI F, AZZI A, BIRRINGER M, et al. Vitamin E: emerging aspects and new directions[J]. Free Radical Biology and Medicine, 2017, 102: 16-36.
[6] YUAN Yisen. Effects of vitamin E and selenium on growth, immunity and antioxidant capacity in ducklings[D]. Master' s thesis. Harbin: Northeast Agricultural University, 2014.
[7] SALARY J, SAHEBI-ALA F, KALANTAR M, et al. In ovo injection of vitamin E on post-hatch immunological parameters and broiler chicken performance[J]. Asian Pacific Journal of Tropical Biomedicine, 2014, 4(S2): S616-S619.
[8] JIANG Shouqun, ZHOU Guilian, LIN Yingcai, et al. Effects of dietary vitamin E level on growth performance, immune function and antioxidant capacity of 22-42 day-old yellow-feathered broilers[J]. Chinese Journal of Animal Nutrition, 2013, 25(2): 289-298.
[9] ZHANG Hongxin, HUANG Renlu, GUO Xiaohu, et al. Effects of vitamin E on performance, egg quality and α-tocopherol content in egg yolk and tissues of laying hens under cold stress[J]. Chinese Journal of Animal Nutrition, 2012, 24(11): 2243-2248.
[10] KAKHKI R A M, BAKHSHALINEJAD R, SHAFIEE M. Effect of dietary zinc and α-tocopheryl acetate on broiler performance, immune responses, antioxidant enzyme activities, minerals and vitamin concentration in blood and tissues of broilers[J]. Animal Feed Science and Technology, 2016, 221: 12-26.
[11] SÜNDER A, FLACHOWSKY G. Influence of high vitamin E dosages on retinol and carotinoid concentration in tissues of laying hens[J]. Archiv Tierernaehrung, 2001(1): 43-52.
[12] ZAPPATERRA M, DESERTI M, MAZZA R, et al. A gene and protein expression study on four porcine genes related to intramuscular fat deposition[J]. Meat Science, 2016, 121: 27-32.
[13] GELDENHUYS W J, LIN L, DARVESH A S, et al. Emerging strategies of targeting lipoprotein lipase for metabolic and cardiovascular diseases[J]. Drug Discovery Today, 2017, 22(2): 352-365.
[14] GUAITA-ESTERUELAS S, GUMÀ J, MASANA L, et al. The peritumoural adipose tissue microenvironment and cancer. The roles of fatty acid binding protein 4 and fatty acid binding protein 5[J]. Molecular and Cellular Endocrinology, 2017, doi:10.1016/j.mce.2017.02.002.
[15] TYRA M, ROPKA-MOLIK K, ECKERT R, et al. H-FABP and LEPR gene expression profile in skeletal muscles and liver during ontogenesis in various breeds of pigs[J]. Domestic Animal Endocrinology, 2011, 40(3): 147-154.

Note: Figure translations are in progress. See original paper for figures.