**Effects of in ovo feeding of vitamin C on post-hatch performance, immune status and DNA methylation-related genes expression in broiler chickens**

Yufei Zhu 1, †, Shizhao Li 2, †, Yulan Duan1, Zhouzheng Ren1, Xin Yang1 and Xiaojun Yang1, \*

***1****College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, People’s Republic of China*

***2*** *Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA*

† *These authors contributed equally to this work.*

\* *Corresponding author: Xiaojun Yang*

*Full Address: College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, PR China*

*Tel: +86-13679287466*

*E-mail:* *yangxj@nwsuaf.edu.cn*

*Short Title:* Vitamin C supplementation and immune status

**Table S1**

Effect of in ovo feeding of vitamin C at E11 on the post-hatch performance of broilers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | Days ofAge | Treatments | SEM | *P*-value |
| NS | VC |
| BW (g) | 1 | 50.3 | 49.4 | 0.42 | 0.476 |
| 21 | 660.1 | 702.2 | 10.91 | 0.067 |
| 42 | 2120.5 | 2261.5 | 41.62 | 0.114 |
| ADFI (g/d) | 1-21 | 51.5 | 50.5 | 0.75 | 0.476 |
| 21-42 | 149.2 | 159.6 | 2.34 | 0.038 |
| 1-42 | 91.8 | 96.8 | 1.23 | 0.067 |
| ADG (g/d) | 1-21 | 29.0 | 31.1 | 0.53 | 0.038 |
| 21-42 | 76.9 | 82.1 | 2.28 | 0.257 |
| 1-42 | 51.8 | 55.3 | 1.04 | 0.114 |
| FCR | 1-21 | 1.77 | 1.63 | 0.032 | 0.019 |
| 21-42 | 1.95 | 1.95 | 0.043 | 0.914 |
| 1-42 | 1.78 | 1.75 | 0.033 | 0.762 |

NS, the normal [saline](file:///D%3A%5CProgram%20Files%5CYoudao%5CDict%5C7.5.0.0%5Cresultui%5Cdict%5C?keyword=saline) group; VC, the vitamin C group; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

On D1, 60 chicks per group; On D21, 54 chicks per group; On D42, 48 chicks per group.

*P*-value is calculated by the non-parametric Mann-Whitney U test (n = 6), and a probability value of *P* < 0.05 and *P* < 0.1 are considered to be statistical significance and trends, respectively.

**Table S2**

Effect of in ovo feeding of vitamin C at E11 on vitamin C content and antioxidant function in plasma of broilers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | Days of age | Treatments | SEM | *P*-value |
| NS | VC |
| Vitamin C content (μg/mL) | 1 | 5.13 | 10.09 | 1.354 | 0.032 |
| 21 | 0.92 | 1.27 | 0.094 | 0.126 |
| 42 | 1.21 | 1.39 | 0.139 | 1.000 |
| T-AOC (U/mL) | 21 | 5.42 | 4.83 | 0.447 | 0.537 |
| 42 | 3.75 | 5.28 | 0.392 | 0.063 |
| GSH-Px/activity unit | 21 | 1684.5 | 1629.1 | 128.56 | 1.000 |
| 42 | 2876.4 | 2932.3 | 220.24 | 0.699 |
| SOD (U/mL) | 21 | 19.82 | 20.78 | 1.346 | 0.931 |
| 42 | 26.58 | 29.97 | 1.320 | 0.240 |
| MDA (nmol/mL) | 21 | 1.10 | 1.05 | 0.058 | 0.589 |
| 42 | 1.09 | 1.08 | 0.094 | 0.662 |

NS, the normal [saline](file:///D%3A%5CProgram%20Files%5CYoudao%5CDict%5C7.5.0.0%5Cresultui%5Cdict%5C?keyword=saline) group; VC, the vitamin C group; T-AOC, total [antioxidant](file:///D%3A%5CProgram%20Files%5CYoudao%5CDict%5C7.5.0.0%5Cresultui%5Cdict%5C?keyword=antioxidant) [capacity](file:///D%3A%5CProgram%20Files%5CYoudao%5CDict%5C7.5.0.0%5Cresultui%5Cdict%5C?keyword=capacity); GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

*P*-value is calculated by the non-parametric Mann-Whitney U test (n = 6), and a probability value of *P* < 0.05 and *P* < 0.1 are considered to be statistical significance and trends, respectively.

**Table S3**

Effect of in ovo feeding of vitamin C at E11 on immune function of broilers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | Days of age | Treatments | SEM | *P*-value |
| NS | VC |
| SI for T lymphocyte | 35 | 1.20 | 1.71 | 0.106 | 0.057 |
| SI for B lymphocyte | 35 | 1.29 | 1.60 | 0.092 | 0.143 |
| Lysozyme activity (U/mL) | 1 | 3.55 | 3.49 | 0.167 | 0.762 |
| 21 | 191.42 | 217.41 | 5.934 | 0.032 |
| 42 | 164.25 | 168.50 | 3.457 | 0.662 |
| IgA (g/L) | 1 | 0.16 | 0.27 | 0.024 | 0.063 |
| 21 | 0.57 | 0.41 | 0.048 | 0.126 |
| 42 | 0.58 | 0.64 | 0.032 | 0.548 |
| IgG (g/L) | 1 | 5.93 | 6.04 | 0.074 | 0.394 |
| 21 | 4.99 | 5.75 | 0.276 | 0.413 |
| 42 | 4.84 | 5.02 | 0.178 | 1.000 |
| IgM (g/L) | 1 | 1.49 | 3.60 | 0.461 | 0.016 |
| 21 | 1.70 | 3.98 | 0.433 | 0.004 |
| 42 | 1.08 | 1.97 | 0.315 | 0.485 |

NS, the normal [saline](file:///D%3A%5CProgram%20Files%5CYoudao%5CDict%5C7.5.0.0%5Cresultui%5Cdict%5C?keyword=saline) group; VC, the vitamin C group; SI, stimulation index; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

*P*-value is calculated by the non-parametric Mann-Whitney U test (n = 6), and a probability value of *P* < 0.05 and *P* < 0.1 are considered to be statistical significance and trends, respectively.