**Effect of in ovo feeding of vitamin C on growth performance, antioxidation and immune function of broiler chickens**

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Short title: The effect of vitamin C on immune function

*animal* journal

Table S1 *Composition and nutrient levels of broiler diets on fed basis*

|  |  |
| --- | --- |
| Items | Content |
| 1-21 d | 22-42 d |
| Ingredient (%) |  |  |
| Corn | 59.74 | 60.06 |
| Wheat | — | 5.40 |
| Soybean meal | 25.50 | 18.50 |
| Cottonseed meal | 2.00 | 3.50 |
| Meat and bone meal | — | 2.00 |
| Fish meal | 1.50 | — |
| DDGS1 | — | 5.00 |
| Limestone | 1.40 | 0.90 |
| NaCl | 0.33 | 0.35 |
| CaHPO4 | 1.50 | 0.60 |
| DL-Methionine | 0.20 | — |
| L-Lysine | — | 0.40 |
| HMA2 | 0.20 | 0.13 |
| Threonine | 0.03 | 0.05 |
| Middling | 5.00 | — |
| Oil | 1.00 | 1.60 |
| Choline chloride | 0.10 | 0.10 |
| Premix3 | 1.50 | 1.50 |
| Total | 100.00 | 100.00 |
| Nutrient levels4 |  |  |
| ME5（MJ/kg） | 12.13 | 12.55 |
| CP6 | 20.00 | 18.50 |
| Total Lysine | 1.17 | 1.00 |
| Total Methionine | 0.49 | 0.40 |
| Total Threonine  | 0.78 | 0.72 |
| Total Tryptophan  | 0.21 | 0.19 |
| NaCl  | 0.33 | 0.35 |
| Ca | 1.00 | 0.90 |
| Total P | 0.66 | 0.60 |
| Available P | 0.42 | 0.38 |

1. Distillers dried grains with soluble.

2. Methionine hydroxyl analogue.

3. The premix provided the following per kg of diets: VA 9 200 IU, VD 3 000 IU, VE 38mg,VK3 3 mg, VB1 3 mg, VB2 10 mg, VB6 5 mg, VB12 0.04 mg, niacinaminde 40 mg, calcium pantothenate 16 mg, folic acid 2 mg, biotin 0.3 mg, Fe 66 mg, Cu 15 mg, Mn 95.4 mg, Zn 96.6 mg, I 0.38 mg, Se 0.41 mg.

4. Nutrient levels were calculated values.

5. Metabolic energy.

6. Crude protein.

Table S2 *Primer sequence of target genes in broiler chickens*

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Accession number | Primer sequences | ProductSize (bp) |
| *β-actin* | X00182.1 | F: ATTGTCCACCGCAAATGCTTC | 114 |
|  |  | R:AAATAAAGCCATGCCAATCTCGTC |  |
| *IL-1β* | NM\_204524 | F: ACAGAGATGGCGTTCGTTCCCGA | 84 |
|  |  | R: TCAGCTCGACGCTGTCGATGT |  |
| *IL-2* | NW\_003763741.1 | F: GCTAATGACTACAGCTTATGGAGCA | 138 |
|  |  | R: TGGGTCTCAGTTGGTGTGTAGAG |  |
| *IL-4* | NW\_003763914.1 | F: AACATGCGTCAGCTCCTGAAT | 98 |
|  |  | R: TCTGCTAGGAACTTCTCCATTGAA |  |
| *IL-6* | NW\_003763664.1 | F: AAATCCCTCCTCGCCAATCT | 106 |
|  |  | R: CCCTCACGGTCTTCTCCATAAA |  |
| *TNF-α* | [AY765397.1](http://www.ncbi.nlm.nih.gov/nucleotide/53854909?report=genbank&log$=nuclalign&blast_rank=2&RID=MF1WCWAG01R) | F: TGTGTATGTGCAGCAACCCGTAGT | 229 |
|  |  | R: GGCATTGCAATTTGGACAGAAGT |  |
| *IFN-γ* | [HQ739082.1](https://www.ncbi.nlm.nih.gov/nucleotide/325515276?report=genbank&log$=nuclalign&blast_rank=2&RID=AJ37TWU6015) | F: ACTGAGCCAGATTGTTTCGATGT | 260 |
|  |  | R: TGCCATTAGCAATTGCATCTCCT |  |
| *DNMT1* | NM\_206952.1 | F: ACAGCCTTCGCCGATTACA | 248 |
|  |  | R: CTCTCCACCTGCTCCACCAC |  |
| *DNMT3A* | NM\_001024832.1 | F: GGATAGCCAAGTTCAGCAAAG | 145 |
|  |  | R: GGGAAGCCAAACACCCTCT |  |
| *DNMT3B* | NM\_001024828.1 | F: GTGCTGTGCCTTGAACATTG | 125 |
|  |  | R: TTCGTAACTTCGGAAACCATT |  |
| *Tet1* | XM\_015278732.1 | F: GGGACAACCGACTGACTCTG | 195 |
|  |  | R: GAGATCCGCGTGGGATGATT |  |
| *Tet2* | NM\_001277794.1 | F: AGGCTATGGTGGTAGCCTCA | 164 |
|  |  | R: GAGCAGCGTGCTTGTGAAAA |  |
| *Tet3* | XM\_015297468.1 | F: ACAGGAACACGGATTTCCCC | 169 |
|  |  | R: TTCTGTGCAAATGGCGATGC |  |
| *Gadd45α* | DQ358721.1 | F: CTTGGCCCAGTTGTTGCTTC | 161 |
|  |  | R: CCGGCACCCACTGATCCATA |  |
| *Gadd45β* | DQ358722.1 | F: CTGCTGCGACAATGACATCC | 175 |
|  |  | R: GCAGTAATTCGCCACCTCGG |  |
| *TDG* | NM\_204750.1 | F: GTTTCGAGAAGGAGGGCGAA | 224 |
|  |  | R: CACGAGGGAACTGAGCACAT |  |
| *MBD4* | NM\_204693.1 | F: GGAAGTACCCCTCTCCCGAA | 158 |
|  |  | R: GTGCAGCTCAATGGGGTACT |  |

IL-1β = interleukin-1β; IL-2 = interleukin-2; IL-4 = interleukin-4; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ; DNMT1 = DNA methyltransferase 1; DNMT3A = DNA methyltransferase 3A; DNMT3B = DNA methyltransferase 3B. Tet1 = ten-eleven translocation protein 1; Tet2 = ten-eleven translocation protein 2; Tet3 = ten-eleven translocation protein 3; Gadd45α = growth arrest and DNA-damage-inducible protein alpha; Gadd45β = growth arrest and DNA-damage-inducible protein beta; TDG = Thymine-DNA glycosylase; MBD4 = methyl-CpG-binding domain protein 4. F = forward; R= reverse.