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Effects of *in ovo* feeding of vitamin E or vitamin C on egg hatchability, performance, carcass traits and immunity in broiler chickens

Farhad Ghane[a], Ali-Ahmad-Alaw Qotbi[a], Marina Slozhenkina[b], Aleksander Anatolievich Mosolov[b], Ivan Gorlov[b], Alireza Seidavi[a], Maria Antonietta Colonna[c], Vito Laudadio[d], and Vincenzo Tufarelli[d]

[a]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran; [b]Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Volgograd, Russia; [c]Department of Agricultural and Environmental Science, University of Bari 'Aldo Moro', Bari, Italy; [d]Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Bari, Italy

ABSTRACT

The effect of *in ovo* feeding of different levels of vitamins C and E on egg hatchability, immune response, growth and carcass traits of broiler chickens were investigated. A total of 672 fertilized eggs were assigned to one of eight experimental groups having three replicates with 28 eggs as follows: (1) negative control (not injected); (2) positive control (injected with 0.2 mL deionized water); (3) vitamin C at 1 mg; (4) vitamin C at 3 mg; (5) vitamin C at 6 mg; (6) vitamin E at 0.5 IU; (7) vitamin E at 0.75 IU; and (8) vitamin E at 1.0 IU. At the end of incubation, the number of chicks hatched, and their individual body weight were recorded. Among hatched birds, a total of 240 mixed chicks were randomly selected (30 subject per group equally shared in three pen floors). Chicks were vaccinated against Avian Influenza, Gumboro, Bronchitis, and Newcastle disease virus. Performance parameters were weekly evaluated until 42 days of age. At days 28 and 42, broiler serum and spleen and Bursa of Fabricius relative weight were assessed as well as on day 42 the carcass traits. From results, *in ovo* injection with 3 mg of vitamin C or 0.75 IU of vitamin E, increased significantly ($p < .05$) the embryos hatchability when compared to the negative control. However, body weight at hatch and growth performance parameters showed no differences among treatments. Similarly, *in ovo* concentrations of vitamins C or E showed no differences on carcass traits, immunity-related organs weight or immune response for anti-Newcastle disease hemagglutination-inhibition and total immunoglobulins against sheep red blood cells (SRBC) when compared to the control groups. Based on findings, it can be concluded that *in ovo* feeding vitamins E and C supported positively chicken embryos hatchability demonstrating the key-role as antioxidant agents; however, further studies are currently being evaluated.

KEYWORDS

Broiler; *in ovo* feeding; hatchability; growth; vitamin C; vitamin E

Introduction

The *in ovo* vaccination for poultry has opened new opportunities also for evaluating *in ovo* feeding.[1,2] Hence, several studies demonstrated positive and encouraging results of *in ovo* feeding of amino acids, minerals, carbohydrates, probiotics, prebiotics and vitamins.[3] Consequently, the advantages this technology provide continue to develop and offer additional benefits to poultry industry.[4] The *in ovo* feeding also produces an exogenous nutrient absorption peak and was reported to improve economic traits, such as body weight gain, conversion factor, carcass characteristics and meat quality, as well as resistance to disease.[5–7]

The high metabolic demand during embryogenesis increases oxidation of lipids from the yolk as well as free reactive oxygen species (ROS), causing lipid peroxidation of cell membranes and cell degradation.[8] To help and prevent this damage, several investigators have evaluated the *in ovo* administration of antioxidants such as vitamin C and vitamin E with encouraging results in the modulation of the antioxidant defence system and immune gene expression.[9,10]

Therefore, the purpose of the present study was to evaluate the effects of *in ovo* feeding of different levels of vitamin C and vitamin E on the egg hatchability,

CONTACT Vincenzo Tufarelli vincenzo.tufarelli@uniba.it Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano, Bari, Italy.

immune response, growth and carcass traits of broiler chickens.

Materials and methods

Hatchery management

Hatching eggs from Ross 308 broiler breeder stocks (at 27 weeks of age), individually weighed, were incubated for 21 days in a commercial hatchery (Jamesway Hatchery Company Inc. PS500 Multi-Stage Controller, Toronto, Canada). The incubation condition were: 37 °C and 70–75% relative humidity from 1 to 18 days of incubation, and 36.5 °C and 75–82% relative humidity from 19 to 21 days of incubation. On day 15 of incubation, the eggs were removed from the incubator, candled and those unfertilized or with dead embryos were discarded. A total of 672 fertilized eggs were washed and sanitized using iodine tincture before injection and were divided into eight experimental groups having three replicates with 28 eggs per replicate, as described below.

Products and experimental design

The vitamin C (Lohmann & Rauscher, 90%, Germany) and vitamin E (DSM, 50%, Switzerland) were purchased, and a solution was prepared using deionized water and injected manually into the egg air chamber by using insulin syringe (Helal Iran Medical Devices Co; Soha, Karaj, Iran) at day 15 of incubation. The experimental dietary groups were inoculated with: (1) negative control (not injected); (2) positive control (injected with 0.2 mL deionized water); (3) vitamin C at 1 mg; (4) vitamin C at 3 mg; (5) vitamin C at 6 mg; (6) vitamin E at 0.5 IU; (7) vitamin E at 0.75 IU; and (8) vitamin E at 1.0 IU. Immediately after the injection, the site was sealed with sterile paraffin and eggs were returned to the incubator. At the end of incubation, the number of chicks hatched were counted and individual body weight assessed. The mortality rate (15–21 days), pecked eggs, dirty eggs, thin layer eggs, breakage-d eggs due to contamination, exploded eggs due to contamination, and so on, and hatchability and ratio of chick weight/egg weight were assessed.

Poultry facility and birds

This experiment was performed under commercial conditions in an air-conditioned poultry house. Among the hatched chickens, a total of 240 mixed chicks were randomly selected (30 birds for each experimental group equally shared in three pen floors) and reared following the animal welfare recommendations of Ethics Committee of Rasht Branch, Islamic Azad University (Rasht, Iran), and care was taken to minimize the number of animals used. A heater was used, and the temperature programmed according to the Ross 308 broilers recommendations (Aviagen, Newbridge, Scotland, UK). Air humidity was kept at 55–65% in the early growing period by spraying water on the floor. The lighting program was 23 h light and 1 h dark. Sanitation principles and health measures for raising chickens were applied. Drinkers were washed and cleaned daily. After each vaccination, 1:1000 multivitamin + electrolytes solution was mixed in the drinking water for 24 hours. Birds were vaccinated against Avian Influenza virus (1st day of age), Gumboro, Infectious Bursal Disease virus (1st day of age), Bronchitis vaccine IB H120 (1st and 12th days of age) and Newcastle Disease virus (NDV), with vaccine B1 and Lasota type respectively (1st and 18th days of age). The rearing conditions of broiler chickens were the same for all groups. Broilers were fed *ad libitum* commercial diets (Table 1) according to their age, and water was also freely provided.

Table 1. Ingredients and chemical composition of basal diet fed to broilers.

Item	Starter (1–15 d)	Grower (16–30 d)	Finisher (31–42 d)
Ingredients (g/kg)			
Corn	611.0	641.0	625.0
Soybean meal (44% CP)	346.0	316.5	316.5
Soybean oil	–	–	20.0
Dicalcium phosphate	15.0	15.0	15.0
Oyster shells	10.0	10.0	10.0
Vitamin-mineral premix[a]	5.0	5.0	5.0
L-Lysine	3.7	3.6	1.7
NaCl	3.5	3.5	3.5
DL-Methionine	2.6	2.5	1.8
Threonine	2.4	2.0	0.3
Termin 8[b]	1.0	1.0	1.0
Nataphous enzyme	–	0.5	0.5
Chemical composition			
Metabolisable energy[c] (kcal/kg)	2,793	2,876	3,004
Crude protein (%)	20.0	19.0	18.9
Lysine (%)	1.26	1.25	1.12
Methionine (%)	0.57	0.55	0.49
Calcium (%)	1.03	1.03	1.03
Phosphorus Available (%)	0.62	0.62	0.62

[a]Supplied per kg: vitamin A, 5,000 IU; vitamin D_3, 500 IU; vitamin E, 3 mg; vitamin K_3, 1.5 mg; vitamin B_2, 1 mg; calcium pantothenate, 4 mg; niacin, 15 mg; vitamin B_6, 13 mg; Cu,3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.
[b]Termin 8 controls pathogens in feed right up to the point of consumption (ANITOX Ltd, EMEA Regional HQ, 80 Main Road, Earls Barton, Northampton, UK).
[c]Metabolizable energy estimated using the Carpenter and Clegg equation[11].

Studied parameters

Body weight and feed intake were weekly measured, whereas average daily feed intake, average daily weight

gain, and feed conversion ratio were calculated for each replicate within each treatment for starter, grower and finisher periods, and for whole study period (1–42 days of age).

On the last day of experiment (day 42), three birds per pen were randomly selected, weighed, and killed by cervical dislocation to evaluate the carcass characteristics. The weight of the entire defeathered carcass and the carcass without head and drumsticks were recorded. Viscera and abdominal fat were then removed, and the carcass yield and relative weight of abdominal fat, meat cuts (breast, drumsticks, wings) and gut were determined. At days 28 and 42, broilers used to evaluate immune response were also killed by cervical dislocation, and the relative weight of organs related to the immune system function (spleen and bursa of Fabricius) were assessed according to[12] Three birds per replicate were randomly chosen to collect blood samples from the brachial vein. Blood serum was pooled per replicate and incubated at room temperature for 1 h, separated by centrifugation, and chilled at −20 °C until analysis. Antibody titer immunoglobulin G2 (IgG2) against NDV (at 18th, 28th and 42nd days of age) were determined using the hemagglutination-inhibition test.[13]

To evaluate the systemic antibody response,[14] birds at 21 and 35 days of age were vaccinated against sheep red blood cells (SRBC) by subcutaneous administration of SRBC suspension in 5% PBS. At 28 and 42 days of age, blood samples collected and pooled per replicate, and total Ig against SRBC determined using a hemagglutination assay in serum 7. In U-bottom microtiter plates, two-fold serial dilutions of heat-inactivated serum (at 56 °C) were added to PBS (0.01 mol/L; pH 7.4) to assess total antibodies, or to phosphate-buffered saline (PBS) with 1.4% 2-mercaptoethanol for assessment of IgG antibodies. All antibody titers were recorded as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titer was determined as the difference between the total titer (IgT) and IgG titer.

Statistical analysis

Data were analyzed by SAS statistical software (version 8[15]) and Tukey's test to compare the means. The total value of any observed treatment-effect and the average test error was the result of the whole population. Before performing data statistical analysis, all data were tested by normality and transformed, if necessary. The results were considered significantly different at $p < .05$.

Results and discussion

In a previous study,[16] presented the possible applications of *in ovo* technology to improve poultry protection against pathogens. During late embryogenesis in poultry, solutions administered into the amniotic fluid (i.e., amino acids, carbohydrates, prebiotics and synbiotics) may be taken up by the embryo, digested and absorbed by the embryonic intestine prior to pipping,[17–21] as well as affecting growth performance and carcass and meat quality traits.[20] *In ovo* feeding of supplemental nutrients may help to overcome the constraint of limited egg nutrients.[1] Other nutrients such as vitamins supplied *in ovo* have also been shown to improve the hatchability and postnatal growth of broiler chickens.

The fast development of chicken embryos is associated with increased production of reactive oxygen metabolites, free radicals and oxidative stress that disrupt the cell membranes by lipoperoxidation.[22] Hence, chicken embryos have antioxidant defence mechanisms to prevent damages to major organs and systems such as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), vitamin C, vitamin E, and a-lipoic acid.[8] *In ovo* injection is considered one of the best strategies to increase the antioxidant defenses of the embryos.[3,8]

Vitamin C, also known as ascorbic acid and L-ascorbic acid, is an essential antioxidant nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters. It is required for the functioning of several enzymes and is essential for immune system function.[23] On the other hand, vitamin E is a group of eight fat-soluble compounds that include four tocopherols and four tocotrienols with antioxidant properties and the cell membrane level and also is involved in controlling gene expression and cell signal transduction.[9,24] Both vitamins have been shown to benefit avian embryonic development during incubation in a dose-dependent manner.

In the present study, a dose of 3 mg of vitamin C or 0.75 IU of vitamin E significantly ($p < .05$) increase the hatchability of embryos when compared with the negative control group. However, body weight at hatch, as well as performance parameters during the growth out of broilers showed no differences ($p > .05$) among treatments (Table 2). Similarly, any of the different *in ovo* concentrations of vitamin C or vitamin E showed no differences on carcass traits (Table 3);

Table 2. Effects of *in ovo* administration of vitamins E and C on egg hatchability and growth performance of broiler chickens.

Item	Hatchability, %	Body weight at hatch, g	Body weight (1–42 d), g	Feed intake (1–42 d), g	Feed conversion ratio (g/g)
NC	92.85[b]	37.61	2232.7	4796.4	2.15
PC	94.04[ab]	37.88	2217.1	4559.1	2.05
Vitamin C 1.0 mg	98.80[ab]	38.08	2224.7	4603.6	2.07
Vitamin C 3.0 mg	100.00[a]	37.92	2390.6	4538.1	1.90
Vitamin C 6.0 mg	96.42[ab]	36.99	2239.4	4478.4	2.00
Vitamin E 0.5 UI/mL	97.61[ab]	37.83	2301.6	4725.0	2.05
Vitamin E 0.75 UI/mL	100.00[a]	37.90	2307.9	4751.0	2.06
Vitamin E 1.0 UI/mL	96.42[ab]	37.24	2296.5	4888.8	2.13
SEM	0.6335	0.1239	36.52	34.89	0.0243
p-value	.0073	.3257	.7660	.6912	.1740

NC = negative control: eggs not injected; PC = positive control: eggs injected with 0.2 mL deionized water; Vitamin C, 1.0 mg; Vitamin C, 3.0 mg; Vitamin C, 6.0 mg; Vitamin E: eggs injected by 0.2 cc soluble Vitamin E, 0.5 IU/mL; Vitamin E, 0.75 IU/mL; Vitamin E, 1.0 IU/mL.
[ab]Mean values bearing different superscripts in the column differ significantly ($p < .05$).

Table 3. Effects of *in ovo* feeding of vitamin E and C on carcass traits and yield in broiler chickens.

Item	NC	PC	Vitamin C 1.0 mg	Vitamin C 3.0 mg	Vitamin C 6.0 mg	Vitamin E 0.5 IU/mL	Vitamin E 0.75 IU/mL	Vitamin E 1.0 IU/mL	SEM	*p*-value
Carcass traits, g										
Live body weight	2180.0	2096.6	2466.7	2550.0	2433.3	2353.3	2410.0	2220.3	49.308	.2278
Empty carcass	1460.1	1406.7	1666.6	1690.0	1606.6	1599.7	1660.6	1483.3	34.708	.2796
Drumsticks	423.0	405.0	489.1	488.2	484.5	432.2	462.4	418.1	12.147	.3400
Breast	519.2	514.3	630.4	612.6	595.1	593.8	600.0	560.04	15.062	.3820
Wings	136.9	125.5	143.7	154.4	154.2	135.7	144.5	138.7	3.894	.6130
Abdominal fat	21.73	22.35	26.28	30.16	24.95	27.97	22.08	17.56	1.654	.2353
Gut,	232.8	205.8	235.5	261.8	252.9	255.1	264.6	234.4	0.271	.7796
Gizzard	54.86	52.26	60.23	67.30	57.93	56.38	60.37	57.81	1.531	.5449
Liver	50.48	43.05	51.71	55.04	51.03	48.91	52.43	45.80	1.675	.8038
Heart	9.36	9.07	10.57	11.48	12.08	10.54	13.00	10.58	0.492	.5369
Carcass yield, %										
Empty carcass	66.07	67.07	67.50	66.82	65.95	67.97	66.67	66.70	8.643	.7513
Drumsticks	28.97	28.79	29.32	28.94	30.15	26.74	28.78	28.25	0.345	.5961
Breast	35.63	36.56	37.83	36.16	37.04	37.12	37.35	37.76	0.292	.4127
Wings	9.38	8.92	8.63	9.06	9.51	8.48	8.99	9.35	0.124	.2641
Abdominal fat	1.01	1.07	1.07	1.35	1.02	1.19	0.92	0.79	0.059	.5564
Gut	15.95	14.63	14.13	15.46	15.74	15.95	16.47	16.12	0.443	.1982
Gizzard	2.52	2.49	2.47	2.66	2.39	2.40	2.50	2.60	0.047	.9431
Liver	2.32	2.05	1.39	2.17	2.10	2.08	2.18	2.06	0.087	.9683
Heart	0.43	0.43	0.43	0.43	0.50	0.45	0.54	0.48	0.023	.7967

NC = negative control: eggs not injected; PC = positive control: eggs injected with 0.2 mL deionized water; Vitamin C, 1.0 mg; Vitamin C, 3.0 mg; Vitamin C, 6.0 mg; Vitamin E: eggs injected by 0.2 cc soluble Vitamin E, 0.5 IU/mL; Vitamin E, 0.75 IU/mL; Vitamin E, 1.0 IU/mL.

Table 4. Effects of *in ovo* feeding of vitamins E and C on immunity-related organs weight and yield of broiler chickens.

Item	NC	PC	Vitamin C 1.0 mg	Vitamin C 3.0 mg	Vitamin C 6.0 mg	Vitamin E 0.5 IU/mL	Vitamin E 0.75 IU/mL	Vitamin E 1.0 IU/mL	SEM	*p*-value
Spleen, g	2.90	2.09	2.89	2.51	2.07	2.77	2.81	2.00	0.1509	.5275
Bursa of Fabricius, g	0.93	0.71	1.56	0.97	1.24	1.09	1.01	1.37	4.0900	.2231
Spleen, %	0.13	0.10	0.12	0.08	0.09	0.12	0.12	0.09	0.0080	.4719
Bursa of Fabricius, %	0.04	0.03	0.06	0.04	0.05	0.05	0.04	0.06	0.0526	.1756

NC = negative control: eggs not injected; PC = positive control: eggs injected with 0.2 mL deionized water; Vitamin C, 1.0 mg; Vitamin C, 3.0 mg; Vitamin C, 6.0 mg; Vitamin E: eggs injected by 0.2 cc soluble Vitamin E, 0.5 IU/mL; Vitamin E, 0.75 IU/mL; Vitamin E, 1.0 IU/mL.

Table 5. Effects of *in ovo* feeding of vitamins E and C on immune response for anti-Newcastle disease (ND) hemagglutination-inhibition (log10) titers and total Sheep Red Blood Cells (SRBC), IgG and IgM (log10) in broilers chickens.

Item	NC	PC	Vitamin C 1.0 mg	Vitamin C 3.0 mg	Vitamin C 6.0 mg	Vitamin E 0.5 IU/mL	Vitamin E 0.75 IU/mL	Vitamin E 1.0 IU/mL	SEM	*p*-value
ND titer, 18 d	2.66	2.00	3.00	2.00	2.00	2.33	2.33	2.33	0.1432	.6697
ND titer, 28 d	2.33	2.33	3.33	3.33	4.66	4.0	4.66	5.66	0.3456	.1508
ND titer, 42 d	5.33	5.00	4.66	5.33	5.33	3.66	6.33	4.66	0.2656	.4347
SRBC (IgT), 28 d	3.33	3.33	2.66	2.66	3.00	2.66	2.33	3.0	0.1250	.4633
SRBC (IgG), 28 d	1.00	1.66	1.00	1.66	1.66	1.00	1.00	1.66	0.0982	.0810
SRBC (IgM), 28 d	2.33	1.66	1.66	1.00	1.33	1.66	1.33	1.33	0.1340	.3934
SRBC (IgT), 42 d	4.0	5.66	6.00	5.66	3.66	5.66	4.00	4.00	0.4368	.7788
SRBC (IgG), 42 d	2.0	3.0	1.66	2.66	2.00	2.00	2.33	2.33	0.2571	.9577
SRBC (IgM), 42 d	2.0	2.66	4.33	3.0	1.66	3.66	1.66	1.66	0.2943	.1327

NC = negative control: eggs not injected; PC = positive control: eggs injected with 0.2 mL deionized water; Vitamin C, 1.0 mg; Vitamin C, 3.0 mg; Vitamin C, 6.0 mg; Vit E: eggs injected by 0.2 cc soluble; Vitamin E, 0.5 IU/mL; Vitamin E, 0.75 IU/mL; Vitamin E, 1.0 IU/mL.

organ weights for immunity-related organs (Table 4); or on the immune response for anti-Newcastle disease hemagglutination-inhibition and total SRBC (Table 5) when compared with the positive or negative control chickens.

Based on findings, it can be concluded that *in ovo* feeding vitamins E and C supported positively chicken embryos hatchability demonstrating their key-role as antioxidant agents; however, further studies are currently being evaluated.

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Disclosure statement

All authors agree to the publication of this manuscript and declare no conflicts of interest.

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ORCID

Farhad Ghane http://orcid.org/0000-0001-7787-9242
Ali-Ahmad-Alaw Qotbi http://orcid.org/0000-0002-4906-9442
Marina Slozhenkina http://orcid.org/0000-0001-9542-5893
Aleksander Anatolievich Mosolov http://orcid.org/0000-0002-3266-9505
Ivan Gorlov http://orcid.org/0000-0002-8683-8159
Alireza Seidavi http://orcid.org/0000-0002-1903-2753
Maria Antonietta Colonna http://orcid.org/0000-0002-2222-2902
Vito Laudadio http://orcid.org/0000-0003-3306-0205
Vincenzo Tufarelli http://orcid.org/0000-0003-0089-4393

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