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INFLUENCE OF *IN OVO* ADMINISTRATION OF SOME WATER-SOLUBLE VITAMINS ON HATCHABILITY TRAITS, GROWTH, CARCASS TRAITS AND BLOOD CHEMISTRY OF JAPANESE QUAILS

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Abstract

A total of 450 fertile Japanese quail eggs were used to determine the impacts of *in ovo* administration of water-soluble vitamins (C, B_6 and B_{12}) on the growth performance, carcass traits, hematological and biochemical blood parameters as well as the immune response of Japanese quails. On the 7th day of incubation, the eggs were allocated to five groups: un-injected, 0.1 ml/egg saline, 1 mg/egg vitamin C, 150 µg/egg vitamin B_6 and 20 µg/egg vitamin B_{12}. The percentage of early embryonic mortality was increased (P≤0.001) in all treated groups *versus* the control group. Chicks that hatched from eggs injected with 1 mg/egg vitamin C exhibited a significantly greater (P≤0.05) live body weight (LBW) than those from the control and saline groups. During 0–2 weeks of age, the chicks hatched from eggs injected with vitamins displayed better feed conversion than the positive or negative controls. *In ovo* injection of vitamins had no significant effect on all carcass traits. *In ovo* injection with vitamins C, B_6 and B_{12} increased plasma total protein and its fractions compared with the control. Plasma levels of total lipids and cholesterol were decreased in chicks hatched from eggs injected with 1 mg/egg vitamin C, 150 µg/egg vitamin B_6 or 20 µg/egg vitamin B_{12} compared with those hatched from control eggs. Plasma T_3 and T_4 were increased in chicks hatched from eggs injected with vitamin C, vitamin B_6 and vitamin B_{12}. The relative weights of the bursa of Fabricius and thymus were significantly (P=0.002 or 0.003) increased in the birds hatched from eggs injected with vitamins compared with those in the control or saline group. Thus, *in ovo* injection of vitamins C, B_6 and B_{12} improved the blood profile and immune response of Japanese quail.

Key words: *in ovo* injection, growth, carcass, blood, immunity, quail

In ovo administration is a strategy to inject some exogenous materials into the amnion through the development of embryo with the objective of promoting positive impacts on hatchability traits, growth rate, feed efficiency, immune functions, and carcass characteristics (Uni and Ferket, 2004). This technique was first used by Sharma and Burmester (1982), when they used this method for the vaccination of turkey eggs against Marek's disease. Recently, the *in ovo* injection methods have been reported by some researchers for administering vitamin C (Sgavioli et al., 2015),

amino acids (Kermanshahi et al., 2015), minerals (Oliveira et al., 2015), vitamins (Salary et al., 2014), royal jelly (Moghaddam et al., 2014) and propolis (Aygun et al., 2012; Aygun, 2016).

A number of nutrients have important physiological, nutritional and immunological functions related to bird's embryogenesis and growth rates. *In ovo* injection of these nutrients may help overcome any constraints imposed by inadequate egg nutrition (Selim et al., 2012). Moreover, *in ovo* administration of nutrients such as vitamins and amino acids may be an alternative strategy for improving hatchability and bird quality at hatch and enhancing the innate immune system (Ohta et al., 2001). Vitamin C (ascorbic acid) has been demonstrated to increase disease resistance and immunoresponsiveness in birds by strengthening the immune system. Also, hatchability percentage was improved when eggs of Pekin ducks were injected with 6 mg/egg of vitamin C on the 20th day of incubation (Nowaczewski et al., 2012). Selim et al. (2012) found that *in ovo* administration of either vitamin E or vitamin C resulted in a significantly greater body weight at hatch and final body weight. Ascorbic acid has also been shown to have an impact on vitamin E by acting as a redox system that returns tocopheroxyl radicals to their tocopherol reduced state (Wilson, 1983).

Pyridoxine (vitamin B_6) deficiency causes early embryonic death and decreases the IgG and IgM response to antibody challenge (Qian et al., 2017). Pyridoxine plays a critical role in the metabolism of carbohydrates, amino acids and fatty acids and exhibits a critical function in energy production through the citric acid cycle (McDowell, 1989). Vitamin B_6 is also involved in the formation of erythrocytes and the activities of insulin, growth hormone and gonadotropic, thyroid, adrenal and sex hormones. Vitamin B_6 is necessary for brain function and development and helps the body to synthesize norepinephrine, serotonin and melatonin hormones (Pond et al., 1995). Since *in ovo* administration of vitamin B_6 at levels of 40, 60, 80 and 120 µg/egg significantly increased the hatchability percentage in Japanese quail (Elsayed et al., 2010). Also, Bhanja et al. (2007) reported that *in ovo* administration of vitamin B_6 to broiler breeder eggs at 100 µg/egg significantly increased the relative weight of chicks at hatch.

Vitamin B_{12} (cobalamin or cyanocobalamin) is a cofactor for a large number of enzymes catalyzing transketolase- and decarboxylation-type reactions (Miller et al., 2005). Cobalamin deficiency in eggs leads to high death rates of embryo just prior to hatching and to chicks expressing polyneuritis. This vitamin is an essential nutrient for many enzyme systems that carry out a number of basic metabolic functions in the body. Vitamin B_{12} plays a key role in normal functions of the nervous system and the brain and in homocysteine metabolism, energy metabolism, blood function, cell division and the immune system (Ermens et al., 2003). The data concerning the effect of *in ovo* administration of water-soluble vitamins, especially vitamin B_6 and B_{12}, on hatchability traits, growth, carcass traits, hematological and biochemical blood parameters and immune response of Japanese quails are scanty, and the previous reports on *in ovo* injection of these vitamins resulted in contradictory conclusions. Therefore, this study was conducted to evaluate the impacts of *in ovo* administration of water-soluble vitamins (C, B_6 and B_{12}) on the growth traits, carcasses, hematological parameters, blood chemistry and immune response of Japanese quails.

Material and methods

Experimental design

The present study was approved by the Ethics Committee on Animal Use of Zagazig University, Egypt. A total of 450 fertile eggs of meat-type Japanese quail with an average weight of 12.5 g were used in this study under a completely randomized design that included five *in ovo* injection treatments (negative-control, positive control – 0.1 ml/egg saline, 1 mg/egg vitamin C, 150 µg/egg vitamin B_6 and 20 µg/egg vitamin B_{12}). Eggs were obtained from a commercial flock at 20 weeks of age over a period of 4 days. Eggs were gathered daily and stored with the large end up in plastic trays at 14°C. Before incubation, eggs were randomly divided into 5 groups as 90 eggs in each group, with 3 replicates (30 eggs each). Eggs were injected on the 7th day of incubation to deposit test material in the air sac through the wide end of the egg.

Egg injection and incubation procedure

Vitamins C, B_6 and B_{12} were kindly supplied by Egyptian International Pharmaceutical Industry Co. (EIPICO) (10th of Ramadan City, Cairo, Egypt) with certified purities of 100.00%, 98.70% and 100.00%, respectively. Vitamin solutions were freshly prepared via dissolution in saline. The saline and vitamin solutions were injected on the 7th day of incubation. Each egg was cleaned, and the large top end of the egg (location of the air cell) was disinfected with ethyl alcohol. The point site of injection was punctured with a hard, thin stylus, and the tested material was injected into the air cell of each egg using a graded insulin syringe. A 1 mL disposable syringe and a 28-gauge, 0.5 inch needle was used for *in ovo* injection. The punctured site was sealed with a non-toxic glue stick. The eggs were then incubated at 37.6°C and 65% RH in an automatic incubator and turned 45° every 1 h. Beginning on the 14th day of incubation, the eggs were maintained at 37.5°C and 70% RH without turning until hatching. After hatching and full feather drying (approximately 8 h post-hatch), 20 chicks from each replicate (60 from each experimental group) with an average body weight close to the group mean were transferred to growing cages until the sixth week of age.

Data collection

Hatchability traits

After hatching, chicks from every treatment group were counted and individually weighed to compute the percentage of hatchability for fertile eggs as well as the chick weight and relative chick weight. Eggs that failed to hatch were opened to count the number of unfertile eggs, and calculate the percentages of early and late embryonic mortality depending on the number of fertile eggs. The stages of embryonic death were estimated as follows: embryos without feathers were classified as early embryonic mortality from 1–9 days of incubation, while embryos with feathers were classified as late embryonic mortality from 10–17 days of incubation.

Growth performance

To evaluate the growth performance and feed utilization, 60 chicks from every treatment group were used in this study. Quails were weighed individually at weekly intervals to calculate LBW. The average body weight gain (BWG) was calculated by subtracting the average initial body weight of the birds for a certain interval from the final body weight in the same interval. Care was taken to collect and weigh the residual feed in the troughs, and these weights were recorded. Feed consumption was calculated by subtracting the weekly residual feed from the offered feed. The feed conversion ratio (FCR) was calculated weekly as the ratio of feed consumption (g) to body weight gain (g).

Carcass traits

At the end of the experiment, four birds from each treatment were selected, weighed, and slaughtered to evaluate the carcass characteristics. The abdominal cavity was subsequently opened, and the edible organs (gizzard, liver and heart) and abdominal fat were removed and weighed. These weights were recorded in grams. Whole eviscerated carcasses were individually weighed, and the dressing percentage or carcass yield was recorded. The whole organ weights were recorded in proportion to the live body weight.

Blood sampling and laboratory analysis

At wk 6, four birds from each treatment were selected and slaughtered to collect the blood samples into heparinized tubes for laboratory analysis. Blood samples were centrifuged at 2146.56 g for 10 min. Plasma samples were transferred to determine plasma protein (g/dl), albumin (g/dl), globulin (g/dl), A/G ratio (1/1), cholesterol (mg/dl), total lipids (mg/dl), AST (aspartate transaminase; U/L), and ALT (alanine transaminase; U/L), by using commercial kits according to the manufacturer's instructions (Spectrum Diagnostics Egyptian Company for Biotechnology, S.A.E.).

Triiodothyronine (T_3; ng/ml) and thyroxin (T_4; ng/dl) as thyroid hormones were analyzed via the RIA method (Akiba et al., 1982). Hemoglobin (Hb; g/dL) was measured in the whole blood samples (Dukes and Schwarte, 1931). Packed cell volume (PCV) percentage was determined according to Schalm (1961). Manual count for total white and red blood cells was carried out using hemocytometer (Campbell, 1995). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) were calculated according to Ritchie et al. (1994).

Measurements of immune competence

Antibody production

Birds were vaccinated with LaSota strain vaccine against Newcastle Disease Virus on 28th day of age and repeated on 35th day of age. Plasma samples were collected after 7 days of the first and the second vaccination on 35th and 42nd days of age to evaluate primary and secondary antibody responses, respectively. The primary and the secondary antibody titers were measured by hemagglutination inhibition test using U-bottom microtiter plates according to Beard et al. (1975).

Relative weight of lymphoid organs

The spleen, bursa of Fabricius and all thymic lobes were removed from the autopsied birds at 42 days of age. Each organ was cleaned of adherent tissue and weighed. Relative weights of organs were calculated as percentages of the pre-slaughter weight.

Statistical analysis

Data (performance, carcasses, blood parameters, etc.) were subjected to the ANOVA procedure for a completely randomized design using the GLM procedures of SPSS version 17.0 (2008). The differences between means were determined using the post hoc Newman-Keuls test ($P<0.05$).

Results

Hatching traits

The hatchability percentage of fertile eggs was altered by *in ovo* injection with different vitamins ($P<0.001$), since the saline-injected group showed a significant decrease ($P<0.001$) in the hatchability percentage compared with the vitamin-injected groups and control (Table 1). Early embryonic mortality was significantly ($P\leq0.001$) increased in all treated groups compared with the control. Notably, the negative control group exhibited the highest hatchability percentage and the lowest early embryonic mortality. In contrast, the positive control group displayed the lowest hatchability percentage and the highest early embryonic mortality. On the other hand, late embryonic mortality was decreased ($P<0.01$) via *in ovo* injection of vitamin B_{12} compared with both control groups. The vitamin C- and vitamin B_6-injected groups showed also a significant improvement in late embryonic mortality compared with the positive control group. The relative chick weight at hatch was significantly ($P<0.001$) improved in the groups treated with vitamins *vs.* negative control.

Table 1. Percentages of hatchability traits, early and late embryonic mortality of Japanese quail chicks as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Traits (%)				Items
Relative chick weight at hatch	Late embryonic mortality	Early embryonic mortality	Hatchability from fertile eggs	
72.04 d	11.11 b	3.33 d	85.56 a	Control
76.64 c	14.45 a	8.89 a	78.67 b	Saline
81.47 ab	10.00 b	5.56 b	84.44 a	Vit. C (1 mg/egg)
83.55 a	5.56 b	5.56 b	82.22 ab	Vit. B_6 (150 µg/egg)
79.68 abc	4.44 c	4.44 c	81.11 ab	Vit. B_{12} (20 µg/egg)
1.02	1.85	1.11	1.89	SEM[1]
<0.001	<0.013	<0.001	<0.001	P-value[2]

Different letters within 1 column are significantly different ($P<0.05$).

[1]SEM = Standard Error Means.

[2]Overall treatment P-value.

Performance and carcass parameters

The performance parameters of the quail chicks subjected to *in ovo* injection with different vitamins are presented in Tables 2 and 3. The live body weight of quails was significantly affected by *in ovo* injection of different vitamins at all ages, except at hatch. Chicks hatched from eggs injected with 10 µg/egg vitamin C exhibited a significantly greater ($P \leq 0.05$) LBW than chicks subjected to the other treatments at all studied ages. Nevertheless, the saline-injected group presented the lowest LBW at all ages. With regard to BWG, there was no significant effect of the *in ovo* injection of different vitamins on BWG at all studied ages, except during 0–2 weeks of age, when the vitamin C- and vitamin B_6-injected groups showed the highest BWG in comparison with the saline group. But differences between these groups and control were not confirmed significantly. Feed intake was only affected significantly ($P<0.001$) during the third experimental period (4–6 weeks of age) when the highest value was recorded in vitamin C-injected group among all treatment groups (Table 3). From 0–2 weeks of age, chicks hatched from eggs injected with the tested vitamins exhibited a better feed conversion ratio than did the positive and negative controls. In contrast, feed conversion was not significantly affected during other experimental periods.

Table 2. Growth performance of Japanese quail as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Body weight gain (g)				Live body weight (g)				Items
0–6-wk	4–6-wk	2–4-wk	0–2-wk	6 wk	4 wk	2 wk	at hatch	
5.14	3.81	7.03	4.57 ab	225.19 b	171.82 ab	73.35 b	9.40	Control
5.04	3.77	6.87	4.49 b	220.84 c	168.08 b	71.90 c	9.10	Saline
5.25	4.06	6.93	4.77 a	230.27 a	173.38 a	76.39 a	9.68	Vit. C (1 mg/egg)
5.20	4.18	6.75	4.67 a	227.78 ab	169.27 b	74.85 b	9.43	Vit. B_6 (150 µg/egg)
5.18	4.16	6.80	4.59 ab	227.02 ab	168.77 b	73.66 b	9.33	Vit. B_{12} (20 µg/egg)
0.05	0.10	0.11	0.04	1.85	1.70	0.65	0.14	SEM[1]
0.099	0.079	0.064	<0.01	<0.01	<0.001	<0.01	0.789	P-value[2]

Different letters within 1 column are significantly different ($P<0.05$).
[1]SEM = Standard Error Means.
[2]Overall treatment P-value.

Table 3. Feed intake and feed conversion of Japanese quail as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Feed conversion (g feed/g gain)				Feed intake (g)				Items
0–6-wk	4–6-wk	2–4-wk	0–2-wk	0–6-wk	4–6-wk	2–4-wk	0–2-wk	
1	2	3	4	5	6	7	8	9
3.50	7.18	2.73	1.77 a	17.98	26.75 ab	19.19	8.00	Control
3.63	6.78	2.71	1.80 a	17.33	25.44 b	18.61	7.95	Saline
3.36	6.75	2.80	1.67 b	18.24	27.37 a	19.40	7.96	Vit. C (1 mg/egg)
3.36	6.75	2.84	1.70 b	17.92	26.59 ab	19.18	8.00	Vit. B_6 (150 µg/egg)
3.40	6.37	2.78	1.70 b	17.54	25.75 b	18.90	7.98	Vit. B_{12} (20 µg/egg)

table 3 – contd.

1	2	3	4	5	6	7	8	9
0.06	0.10	0.03	0.02	0.032	0.44	0.15	0.02	SEM[1]
0.091	0.089	0.077	0.005	0.077	<0.001	0.33	0.076	P-value[2]

Different letters within 1 column are significantly different (P<0.05).
[1]SEM = Standard Error Means.
[2]Overall treatment P-value.

Table 4. Carcass traits (%) of Japanese quail chicks as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Traits (%)						Items
Dressing	Giblets	Heart	Liver	Gizzard	Carcass	
81.25	4.73	0.91	2.29	1.54	76.52	Control
80.06	4.39	0.92	1.90	1.56	75.67	Saline
80.67	4.45	0.92	2.08	1.47	76.10	Vit. C (1 mg/egg)
80.71	4.55	0.93	2.01	1.63	76.07	Vit. B_6 (150 µg/egg)
80.54	4.40	0.95	1.99	1.48	75.96	Vit. B_{12} (20 µg/egg)
0.19	0.12	0.03	0.11	0.05	0.45	SEM[1]
0.121	0.221	0.452	0.890	0.077	0.076	P-value[2]

[1]SEM = Standard Error Means.
[2]Overall treatment P-value.

The Japanese quail carcass characteristics affected by the *in ovo* injection of different vitamins are illustrated in Table 4. The *in ovo* injection of the vitamins had no significant effect on any of the studied carcass traits (carcass, gizzard, liver, heart, giblet and dressing percentages).

Hematological parameters

The hematological parameters affected by the *in ovo* injection of different vitamins are presented in Table 5. Insignificant differences in MCHC were found between the groups treated with different vitamins and the control groups. However, significant increments in WBCs (P<0.01), RBCs (P<0.01), PCV (P=0.004) and Hb (P=0.001) were noted in the chicks hatched from eggs injected with vitamin B_6 and B_{12} compared with the negative and positive control groups. Moreover, vitamin C administration elevated the WBC count (P=0.003) to a higher level in comparison to both control groups, whereas its effect on RBCs (P=0.002) and PCV (P=0.004) was only significant in comparison with the positive control. The highest MCV and MCH values were recorded in the chicks hatched from eggs injected with vitamin B_6 among all experimental groups.

Blood constituents

Plasma total protein and its fractions

The levels of plasma total protein and its fractions (albumin, globulin and A/G ratio), as affected by *in ovo* injection of different water-soluble vitamins, are illus-

trated in Table 6. The results showed that *in ovo* injection with 1 mg/egg vitamin C, 150 µg/egg vitamin B_6 and 20 µg/egg vitamin B_{12} significantly (P<0.01) increased plasma total protein in comparison to both control groups. While plasma albumin and globulin were significantly (P<0.05) increased due to different vitamins administration compared with negative and positive control, respectively. A/G ratio was not (P>0.05) affected by various *in ovo* injection treatments.

Table 5. Hematological parameters of Japanese quail chicks by *in ovo* injection with vitamins C, B_6 and B_{12}

Parameters								Items
RDW (%)	MCHC (g/dL)	MCH (pg)	MCV (fL)	Hb (g/dL)	PCV (%)	RBCs ($\times10^6$/mm^3)	WBCs ($\times10^3$/mm^3)	
9.50 b	26.45	47.45 bc	178 a	11.80 b	44.50 bc	2.50 cd	267 b	Control
13.30 ab	29.65	47.10 bc	166 b	12.25 b	40.90 c	2.45 d	273 b	Saline
15.25 a	28.60	49.80 a	174 a	13.40 b	46.80 b	2.65 c	286 a	Vit. C (1 mg/egg)
10.95 b	28.20	50.25 a	178 a	15.50 a	55.00 a	3.10 b	286 a	Vit. B_6 (150 µg/egg)
12.40 ab	28.60	45.70 c	158 c	15.45 a	53.90 a	3.45 a	289 a	Vit. B_{12} (20 µg/egg)
0.98	0.89	0.21	1.18	0.30	1.75	0.09	2.51	SEM[1]
<0.05	0.741	<0.05	<0.01	<0.001	<0.01	<0.01	<0.01	P-value[2]

Different letters within 1 column are significantly different (P<0.05).
[1]SEM = Standard Error Means.
[2]Overall treatment P-value.

Table 6. Plasma constituents and thyroid hormones of Japanese quail chicks as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Blood chemistry[1]									Items
T_3/T_4 ratio	T_4 (µg/dl)	T_3 (ng/ml)	CHO (mg /dl)	TL (mg /dl)	A/G ratio	GLO (a) g/dl	ALB (Ag/dl)	TP (g/dl)	
0.252	4.13 c	1.04 bc	175 a	713 a	0.59	2.91 a	1.72 c	4.63 b	Control
0.252	4.05 c	1.02 c	176 a	710 a	0.71	2.67 b	1.89 b	4.55 c	Saline
0.249	4.46 a	1.11 a	162 b	671 b	0.63	3.06 a	1.90 b	4.96 a	Vit. C (1 mg/egg)
0.245	4.45 a	1.09 a	153 b	672 b	0.65	3.01 a	1.96 a	4.96 a	Vit. B_6 (150 µg/egg)
0.242	4.23 b	1.07 ab	155 b	670 b	0.65	2.99 a	1.94 a	4.92 a	Vit. B_{12} (20 µg/egg)
0.05	0.08	0.02	6.48	9.25	0.02	0.04	0.02	0.01	SEM[1]
0.085	0.003	0.045	<0.001	<0.001	0.089	<0.05	<0.05	<0.01	P-value[2]

Different letters within 1 column are significantly different (P<0.05).
[1]TP: total protein, ALB: albumin, BLO: globulin, TL: total lipid, CHO: cholesterol, T3: triiodothyronine, T_4: Thyroxin.
[2]SEM = Standard Error Means.
[3]Overall treatment P-value.

Total lipids and cholesterol

The average total lipids and cholesterol values, as affected by *in ovo* injection of different vitamins, are illustrated in Table 6. Plasma levels of total lipids and cho-

lesterol were significantly (P<0.001) decreased in chicks hatched from eggs injected with 1 mg/egg vitamin C, 150 µg/egg vitamin B_6 and 20 µg/egg vitamin B_{12} compared with chicks hatched from the negative or positive control groups.

Thyroid hormones

Table 6 summarizes the plasma levels of T_3 and T_4 and the T_3/T_4 ratio, as affected by *in ovo* injection of different vitamins (C, B_6 and B_{12}). Plasma T_3 and T_4 were significantly (P=0.045 or 0.003) increased in chicks hatched from eggs injected with 1 mg/egg vitamin C and 150 µg/egg vitamin B_6 compared with those hatched from both control groups. Vitamin B_{12} administration exhibited the same trend only for plasma T_4 concentrations, while plasma T_3 levels were significantly higher in chicks hatched from this group only when compared with those hatched from positive control group. In contrast, there were no significant differences in the T_3/T_4 ratio among the various experimental groups.

Measurements of immune competence

Antibody production

The effects of *in ovo* injection of different water-soluble vitamins on antibody production against NDV (humeral immunity) in Japanese quail chicks are presented in Table 7. The primary and secondary total antibody titers estimated 7 days after the first and second immunization with NDV, respectively, were significantly (P<0.001) improved in the chicks hatched from eggs injected with different vitamins compared with both the negative and positive controls. Higher levels of primary and secondary antibody production were recorded in chicks hatched from eggs injected with vitamins B_6 and B_{12} in comparison with vitamin C.

Table 7. Humoral immunity against NDV and relative lymphoid organ percentages of Japanese quail chicks as affected by *in ovo* injection with vitamins C, B_6 and B_{12}

Relative lymphoid organ percentages			Humoral immunity		Items
spleen (%)	bursa (%)	thymus (%)	total secondary antibody titers (log_2)	total primary antibody titers (log_2)	
0.111	0.078 bc	0.465 b	5.01 c	3.67 c	Control
0.110	0.074 c	0.455 b	4.33 d	3.33 c	Saline
0.112	0.080 b	0.487 a	6.67 b	5.33 b	Vit. C (1 mg/egg)
0.115	0.086 a	0.497 a	7.67 a	6.67 a	Vit. B_6 (150 µg/egg)
0.113	0.084 a	0.488 a	7.33 a	6.33 a	Vit. B_{12} (20 µg/egg)
0.002	0.001	0.01	0.37	0.33	SEM[1]
0.875	0.003	0.002	<0.001	<0.001	P-value[2]

Different letters within 1 column are significantly different (P<0.05).
[1]SEM = Standard Error Means.
[2]Overall treatment P-value.

Relative weight of lymphoid organs

The effect of *in ovo* administration of chosen water-soluble vitamins on the relative weight of the lymphoid organs of Japanese quail is shown in Table 7. The weights of thymus and bursa were significantly increased (P=0.002 or 0.003) in birds hatched from eggs injected with tested vitamins compared with the un-injected (except bursa in vitamin C treated group) or saline-injected group. In contrast, no significant (P>0.05) differences were found among experimental treatments in the relative weight of the spleen.

Discussion

In the present study, the negative control group exhibited the highest hatchability percentage and the lowest early embryonic mortality. While, the positive control group displayed the lowest hatchability percentage and the highest early and late embryonic mortality. Results concerning the *in ovo* injection of vitamin C were in agreement with those obtained by Selim et al. (2012) who found that *in ovo* injection of saline solution with or without vitamin C reduced the hatchability rate compared with the un-injected group. The greatest reported improvement in hatchability percentage was achieved when eggs of Pekin ducks were injected with 6 mg/egg of vitamin C on the 20th day of incubation (Nowaczewski et al., 2012). The decrease in hatchability observed after *in ovo* injection of saline with or without vitamin C may be due to the different osmolality of the injected solution, which is a key factor affecting hatchability in turkeys (Ferket et al., 2005). Elsayed et al. (2010) reported that *in ovo* administration of vitamin B_6 at levels of 40, 60, 80 and 120 µg/egg significantly increased the hatchability percentage in Japanese quail. El-Kholy (2013) found that *in ovo* administration of 0.1 ml/egg of saline solution (positive control) and 150 µg/egg of vitamin B_6 significantly (P≤0.05) decreased the hatchability percentage of fertile eggs and early embryonic mortality compared with the untreated group.

With regard to BWG, there was no significant effect of the *in ovo* injection of different vitamins on BWG at all studied ages, except during 0–2 weeks of age, when the vitamin C- and vitamin B_6-injected groups showed the highest BWG. The greater body weight of the vitamin C-injected group may be due to the important function of vitamin C through incubation in the subsequent growth of broiler chicks to market weight, as previously reported (Zakaria et al., 1998). Importantly, male chicks display a limited capacity for vitamin C biosynthesis (Hornig and Frigg, 1979). In addition, the rapid growth rates of male broilers are considered to represent a stressful condition, resulting in the need to increase vitamin C requirements. The obtained findings were consistent with the results of Zakaria (2001), who found that *in ovo* administration of 3 mg of vitamin C on the 15th day of incubation results in a greater body weight in males, but no significant difference in body weight was observed in female broiler chicks. Our results concerning the *in ovo* injection of vitamin B_6 were in agreement with those obtained by Bhanja et al. (2007), who injected broiler eggs with vitamin B_6 (100 µg/egg) dissolved in 0.5 ml of sterile water and showed no sig-

nificant effect of vitamin B_6 administration on LBW at 14 days of age. However, contradictory results have been reported by El-Kholy (2013), who found that injection of Japanese quail eggs with vitamin B_6 at 100–120 µg/egg significantly affected LBW at the 2nd and 3rd week of age and body weight gain during the period from 1 to 2 weeks of age. These findings contrast with our results, Bhanja et al. (2007) injected broiler breeder eggs with vitamin B_6 (100 µg/egg) dissolved in 0.5 ml of sterile water and showed that LBW significantly increased at 28 days of age. Selim et al. (2012) found that *in ovo* administration of either vitamin E or vitamin C resulted in a significantly greater ($P \leq 0.05$) LBW at hatch and final LBW.

The present study showed that feed intake was the highest from 4 to 6 weeks of age in the vitamin C-injected group among all treatments. From 0 to 2 weeks of age, chicks hatched from eggs injected with the tested vitamins exhibited better feed conversion ratio than did the positive and negative controls. The results obtained herein were partially in agreement with those obtained by Selim et al. (2012) who found that *in ovo* administration of vitamin C increased the feed intake of ducks apart from feed intake through the finishing period in females only, compared with un-injected and sham groups. Furthermore, *in ovo* administration of vitamin C caused a significant decrease in the feed conversion ratio of male ducks apart from feed conversion ratio through the starting period, but no significant change in females (except during the finishing period, when the feed conversion ratio significantly decreased compared with control). The present study was supported by a study by Bhanja et al. (2007) who demonstrated that injection of vitamin B_6 into broiler breeder eggs (100 µg/egg) had no significant effect on feed conversion. Similarly, El-Kholy (2013) found that the injection of Japanese quail eggs with 100 or 150 µg/egg of vitamin B_6 improved the feed conversion ratio from 0 to 2 weeks of age. The *in ovo* injection of the vitamins had no significant effect on any of the studied carcass traits. These results agreed with those obtained by Elsayed et al. (2010), who injected Japanese quail eggs with vitamin B_6 at levels of 40, 60, 80 and 120 µg/egg and found that the heart, gizzard and giblet percentages were not affected significantly by vitamin B_6 administration at any of the tested levels. Similarly, El-Kholy (2013) observed insignificant differences in the carcass, gizzard, liver, heart, total giblet and dressing percentages following *in ovo* injection of Japanese quail with vitamin B_6 at 100 and 150 µg/egg.

In the present study, a significant increment in WBCs, RBCs, PCVand Hb was noted in the chicks hatched from eggs injected with vitamin B_6 and B_{12} compared with the control groups. The highest MCV and MCH values were recorded in the chicks hatched from eggs injected with vitamin B_6 among all experimental groups. Red cell distribution width (RDW) was increased in all treated groups compared with the control group. El-Kholy (2013) observed that *in ovo* injection of 150 µg/egg of vitamin B_6 into quail eggs did not significantly affect the packed cell volume, hemoglobin content or MCHC. Sgavioli et al. (2013) found that vitamin C injected into eggs did not influence the hematological parameters of chicks compared with uninjected controls. However, chicks from eggs injected with 4% vitamin C showed a higher total RBC count and levels of hematocrit and Hb when incubation was performed at hot temperatures. Pires et al. (2011) did not report any impact of vitamin C injection on the erythrocyte characteristics of broiler chicks. Vitamin C improves

the absorption of minerals such as iron, consequently increasing both the number of HGB and RBC levels (Moura and Pedroso, 2003). The developing embryo mainly depends on the egg composition, which may therefore affect hatching rates and chick quality, both during and after hatching (Finkler et al., 1998). Thus, intra-egg administration of nutrients such as vitamins and amino acids may be an alternative strategy for manipulating the quality of chicks and their performance after hatching. If vitamin C is an anti-stressor and enhances the performance of the bird (Mahmoud et al., 2004), it is possible that intra-egg injection of vitamin C may be beneficial to embryos under thermo-neutral or heat-stress conditions (Nowaczewski et al., 2012).

Our results showed that *in ovo* injection with chosen vitamins significantly increased plasma total protein and its fractions. Concerning the effect of vitamin B_6 (150 µg/egg), our results were in agreement with those obtained by Elsayed et al. (2010), who injected Japanese quail eggs with vitamin B_6 at levels of 40, 80, 100 and 120 mg/egg and recorded a significant increase in total protein, albumin and globulin levels in chicks hatched from eggs injected with 100 mg/egg vitamin B_6. In this regard, El-Kholy (2013) found that treatment with vitamin B_6 at 150 mg/egg significantly ($P<0.05$) increased plasma total protein and globulin contents, whereas plasma albumin and the A/G ratio were not significantly affected by *in ovo* injection treatments with different vitamins, consistent with our results. Goel et al. (2013) reported that the serum protein level was higher ($P<0.01$) after vitamin B_2, B_6 or E treatment. The higher serum protein level in vitamin B_2-injected birds may be due to the ability of vitamin B_2 to break down protein, carbohydrates and fat by acting as a cofactor for numerous enzymes.

Plasma levels of total lipids and cholesterol were decreased in vitamin-treated groups in comparison to control groups. These findings were in agreement with the results of El-Kholy (2013), who concluded that *in ovo* injection of Japanese quail eggs with 100 or 150 mg/egg vitamin B_6 decreased total lipid levels and that *in ovo* administration of 100 mg/egg vitamin B_6 decreased plasma cholesterol. Elsayed et al. (2010) injected Japanese quail eggs with vitamin B_6 at levels of 40, 80 and 120 mg/egg and found that serum total lipids were not significantly affected by vitamin B_6 treatment but that the cholesterol concentration was positively correlated with increasing vitamin B_6 levels. The first few days after hatching are a critical period for the survival and development of neonates in poultry species due to considerable energy catabolism (Ebrahimnezhad et al., 2011). Orlov (1987) confirmed that the percentage of a feed additive that passes from the body of the bird to the egg is approximately 25–30%, while the rest is allocated to the body of the bird. Therefore, *in ovo* injection is a quick route for transferring nutrient compounds such as amino acids, vitamins and glucose directly to the developing embryo (Al-Asady, 2006; Mahmood, 2010).

In the current study, plasma T_3 and T_4 were increased due to tested vitamins treatment, these results were similar to those obtained by Elsayed et al. (2010), who reported that the serum T_3 concentration was significantly ($P<0.01$) elevated in quails hatched from eggs injected with 120 µg B_6/egg.

Higher levels of primary and secondary antibody production were recorded in the present study for chicks hatched from eggs injected with vitamins B_6 and B_{12} in

comparison with vitamin C. Our results were in agreement with those obtained by Selim et al. (2012) who reported that *in ovo* administration of vitamin C resulted in a significant increase in the geometric mean of the 1st estimate for the antibody titers of male ducks and the 1st and 2nd estimates for the antibody titers of female ducks compared with sham and un-injected control groups. Moreover, Blalock et al. (1984) investigated the humoral immune response of chickens that were moderately deficient in vitamin B_6 and found that marginal pyridoxine deficiency caused significant reductions in the relative levels of IgM and IgG and antibody levels against sheep red blood cells (SRBCs) through the peak and degradation phases of the primary responses. Similarly, primary and secondary total antibody titers against NDV were found to be enhanced in chicks hatched from eggs injected with vitamin B_6 at 150 µg/egg (El-Kholy, 2013). Pyridoxine is functionally important as pyrodoxal phosphate in the transformation of amino acids and supporting the synthesis of proteins required for immune responses (Hossain et al., 1998).

The weights of thymus and bursa were significantly increased in birds hatched from eggs injected with vitamins compared with control groups. These results are harmonized with the findings of El-Kholy (2013) who observed that the injection of eggs with vitamin B_6 significantly increased the weights of the thymus and bursa but did not significantly affect the spleen weight. Goel et al. (2013) found that the relative weight of the male bursa was greater in vitamin B_1 (18 µg) or B_2 (36 µg)-injected chicks but that the weight of thymus was greater in vitamin B_6 (35 µg)-injected chicks. In our study, the weights of the bursa and thymus were higher under vitamin B_6 treatment, in agreement with the earlier study of Blalock et al. (1984) who observed that vitamin B_6 deficiency caused a significant reduction in the concentrations of antibodies against sheep red blood cells and in the relative concentrations of IgG and IgM through the degradation and peak phases of the primary response. The results of the current study were in line with those of Bakyaraj et al. (2012), who reported that bursa weight at hatch was improved in vitamin-injected chicks. The bursa is involved in the production of antibodies, thus affecting humoral immunity, whereas the thymus plays a key role in cellular immune responses. In the present study, birds with greater bursa weights also exhibited greater thymus weights, indicating improved cellular immunity.

Conclusion

Our findings suggested that early administration of some nutrients, such as vitamins C, B_6 and B_{12}, through *in ovo* administration can be regarded as a possible strategy for improving the growth traits, lipid profile and immune system of Japanese quails. Where, chicks that hatched from eggs injected with vitamin C exhibited a significantly greater LBW than those from positive and negative controls. Also, *in ovo* injection with vitamins increased plasma total protein and its fractions as well as the weights of the bursa of Fabricius and thymus, but decreased plasma levels of total lipids and cholesterol in comparison with the control or saline group.

References

Akiba Y., Jensen L.S., Bart C.R., Kraeling R.R. (1982). Plasma estradiol, thyroid hormones and liver lipids determination in birds. J. Nutr., 112: 299–308.

Al-Asady A.N. (2006). Effect of injecting hatching egg with nutritional solutions and early feeding on some productive and physiological traits of broiler chicken. M.Sc. Thesis, College of Agriculture, University of Baghdad.

Aygun A. (2016). The effects of *in ovo* injection of propolis on egg hatchability and starter live performance of Japanese quails. Rev. Bras. Cienc. Avic., 18: 83–89.

Aygun A., Sert D., Copur G. (2012). Effects of propolis on eggshell microbial activity, hatchability, and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. Poultry Sci., 91: 1018–1025.

Bakyaraj S., Bhanja S.K., Majumdar S., Dash B. (2012). Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. J. Sci. Food Agric., 92: 313–320.

Beard C.W., Hopkins S.R., Hammond J. (1975). Preparation of Newcastle disease virus hemagglutination-inhibition test antigen. Avian Dis., 19: 692–699.

Bhanja S.K., Mandal A.B., Agarwal S.K., Majumdar S., Bhattacharyya A. (2007). Effect of *in ovo* injection of vitamins of the chick weight and post-hatch growth performance in broiler chickens. Proc. 16th European Symposium on Poultry Nutrition. Strasbourg, France, 26–30.08.2007.

Blalock T.L., Thaxton J.P., Garlich J.D. (1984). Humoral immunity in chicks experiencing marginal vitamin B-6 deficiency. J. Nutr., 114: 312–322.

Campbell T.W. (1995). Avian Hematology and Cytology. 2nd ed., Iowa State University Press, Ames, Iowa, USA.

Dukes H.H., Schwarte Z.L.H. (1931). The hemoglobin content of the blood of the fowl. Am. J. Physiol., 96: 89–92.

Ebrahimnezhad Y., Salmanzadeh M., Aghdamshahryar H., Beheshti R., Rahimi H. (2011). The effect of *in ovo* injection of glucose on character of hatching and parameters of blood in broiler chickens. Ann. Biol. Res., 2: 347–351.

El-Kholy M.S. (2013). Physiological studies on Japanese quail as affected by some nutritional treatments. PhD Thesis, Fac. Agric., Zagazig Univ., Egypt.

Elsayed M.A.,Wakwak M.M., Mahrose K.H.M. (2010). Effect of pyridoxine injection in Japanese quail eggs on hatchability, performance and some physiological parameters. Isotope Rad. Res., 42: 109–123.

Ermens A.A., Vlasveld L.T., Lindemans J. (2003). Significance of elevated cobalamin (vitamin B_{12}) levels in blood. Clin. Bioch. 36: 585–590.

Ferket P., De J., Ghane A., Uni Z. (2005). Effects of *in ovo* feeding solution osmolality on hatching turkey. Poultry Sci., 84: 117–121.

Finkler M.S., Van Orman J.B., Sotherland P.R. (1998). Experimental manipulation of egg quality in chickens: Influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. J. Comp. Physiol. B., 168: 17–24.

Goel A., Bhanja S.K., Pande V., Mehra M., Mandal A. (2013). Effects of *in ovo* administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. Indian J. Anim. Sci., 83: 916–921.

Horning M.P., Frigg M. (1979). Effects of age on biosynthesis of ascorbate in chicks. Arch. Geflugelkd., 43: 108–112.

Hossain S.M., Barreto S.L., Bertechini A.G., Rios A.M., Silva C.G. (1998). Influence of dietary vitamin E level on egg production of broiler breeders, and on the growth and immune response of progeny in comparison with the progeny from eggs injected with vitamin E. Anim. Feed Sci. Techol., 78: 307–317.

Kermanshahi H., Daneshmand A., Emami N.K., Tabari D.G., Doosti M., Javadmanech A., Ibrahim S.A. (2015). Effect of *in ovo* injection of threonine on Mucin2 gene expression and digestive enzyme activity in Japanese quail (*Coturnix japonica*). Res. Vet. Sci., 100: 257–262.

Mahmood S.M.A. (2010). The effect of injecting hatching eggs with different concentrations of biotin on the embryonic development, productive and physiological traits of the broiler chicken. M.Sc. Thesis. College of Agriculture, University of Baghdad.

Mahmoud K.Z., Edens F.W., Eisen E.J., Havenstein G.B. (2004). Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (*Gallus gallus domesticus*) subjected to cyclic heat stress. Comp. Biochem. Physiol., 137: 35–42.

McDowell LR. (1989). Vitamins in animal nutrition. San Diego, CA, Academic Press Inc.

Miller A., Maya K., Ronit A., Yanina G. (2005). Vitamin B_{12}, demyelination, remyelination and repair in multiple sclerosis. J. Neur. Sci., 233: 93–97.

Moghaddam A.A., Borji M., Komazani D. (2014). Hatchability rate and embryonic growth of broiler chicks following *in ovo* injection royal jelly. Brit. Poultry Sci., 55: 391–397.

Moura L.C., Pedroso M.A. (2003). Anemia ferroprivanagestação. Ver. Enferm. Unisa., 4: 70–75.

Nowaczewski S., Kontecka H., Krystianiak S. (2012). Effect of *in ovo* injection of vitamin C during incubation on hatchability of chickens and ducks. Folia Biol., 60: 93–97.

Ohta Y., Kidd M.T., Ishibashi T. (2001). Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos and chicks after *in ovo* administration of amino acids. Poultry Sci., 80: 1430–1436.

Oliveira T.F.B., Bertechini A.G., Bricka R.M., Kim E.J., Gerard P.D., Peebles E.D. (2015). Effects of *in ovo* injection of organic zinc, manganese, and copper on the hatchability and bone parameters of broiler hatchlings. Poultry Sci., 94: 2488–2494.

Orlov M.V. (1987). Biological Control in Incubation. 3rd ed. Moscow, Rosselhozizdat Russcellezgat.

Pires D.L., Sgavioli S., Malheiros E.B., Boleli I.C. (2011). Acidoascórbico in ovo e jejum-sobreparâmetrossanguíneos. In: XXII Reunión de la Asociación Latinoamericana de Producción Animal – ALPA, Montevideo, Uruguai.

Pond W.G., Church D.C., Pond K.R. (1995). Folic acid. In: Basic Animal Nutrition and Feeding, 4th ed. Wiley, New York, NY.

Qian B., Shen S., Zhang J., Jing P. (2017). Effects of vitamin B_6 deficiency on the composition and functional potential of T cell populations. J. Immun. Res., 4: 1–12.

Ritchie B.W., Harrison G.J., Harrison L.R. (1994). Avian Medicine: Principles and Application. Wingers Publishing Inc., Lake Worth, Florida, USA.

Salary J., Sahebi-Ala F., Kalantar M., Matin H.R.H. (2014). *In ovo* injection of vitamin E on post-hatch immunological parameters and broiler chicken performance. Asian Pac. J. Trop. Biomed., 4: 616–619.

Schalm O.W. (1961). Veterinary Hematology. Lea and Febiger, Philadelphia USA, pp. 165–187.

Selim Sh.A., Gaafar K.M., El-ballal S.S. (2012). Influence of *in ovo* administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emir. J. Food Agric., 24: 264–271.

Sgavioli S., Matos Júnior J.B., Borges L.L., Praes M.F.F.M., Morita V.S., Zaniratto G.L. (2015). Effects of ascorbic acid injection in incubated eggs submitted to heat stress on incubation parameters and chick quality. Braz. J. Poultry Sci., 17: 181–190.

Sgavioli S., Thimotheo M., Praes M.F.F.M., Silva V.K., Alves M.F.R., Boleli I.C. (2013). Effect of ascorbic acid injected on incubation and respiratory hematological parameters. Ars. Veterinaria, 29: 118–125.

Sharma J.M., Burmester B.R. (1982). Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. Avian Diseases, 26: 134–149.

Uni Z., Ferket R.P. (2004). Methods for early nutrition and their potential. World's Poultry Sci. J., 60: 101–111.

Wilson R.L. (1983). Free radical protection: Why vitamin E not vitamin C, b-carotene or glutathione? Biology of Vitamin E. Ciba Foundation Symposium 101. London, UK, The Pitman Press, pp. 19–37.

Zakaria A.H. (2001). Effect of ascorbic acid treatment during egg incubation on pre- and post-hatching development of broiler chickens. J. Damascus Univ. Agri. Sci., 17: 118–130.

Zakaria A.H., Al-latif A.A., Al-Anezi M.A. (1998). Effect of ascorbic acid on embryonic development, hatch time and growth of extended delayed placement of broiler chickens. Arch. Geflugelkd., 62: 11–15.

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