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Effects of *in ovo* administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens

AKSHAT GOEL[1], SUBRAT KUMAR BHANJA[2], VEENA PANDE[3], MANISH MEHRA[4] and ASITBARAN MANDAL[5]

Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

In ovo administration of vitamins A (105IU), B1 (18µg), B2 (36µg), B6 (35µg) or E (1.4IU) in eggs (600) were carried out on 14th day of incubation to study post-hatch performance in broiler chickens. Per cent hatchability in vitamins injected eggs ranged from 73.0 to 84.0 compared to 84.0 in un-injected control. Day-old chick weight was lower in vitamin B6 injected eggs. Body weight of male chicks did not differ due to vitamin administration till 28-day post-hatch, however at 14-day female chicks receiving vitamins A, B1, B2 or E injections weighed higher compared to control chicks. At marketable age (42 d) both male and female birds of vitamin B1 or B2 had higher body weight than control birds. Relative weight of bursa was higher in vitamins B1, B2 or E, while thymus weight was higher in vitamins A, B6 or E injected chicks. At 42nd day post-hatch, humoral immune response (anti SRBC, HA titer) was better in vitamin B1 injected chicks but antibody titer to Newcastle disease (ND) vaccine (log 10 titer) was higher in vitamin E injected chicks. Serum glucose level was higher in vitamins A, B2 or E while B2, B6 or E injected chicks had higher serum protein. From the above study it may be concluded that *in ovo* feeding of vitamin B1 and B2 can improve growth while vitamin A, B1, B6 or E can modulate immunity in broiler chickens.

Key words: Blood biochemicals, Broiler chicken, *In ovo* feeding, Post-hatch performance, Vitamins

Nutrient utilization starts from the very first day of incubation, where both albumen and yolk of egg nurture developing embryo. Being a close system, proper nutrient supply to the developing embryo is of utmost importance. Maternal nutrition is the only source of vitamins for egg but ignorance in breeder's diet or use of poor quality vitamins results in deficiencies leading to embryonic mortality during middle or late incubation period. Besides, deficiency of vitamin A results in reduced cellular immune responses (Lessard *et al.* 1997) while vitamin E acts as antioxidant in reducing cellular free radical damage and improves humoral immune responses (Hossain *et al.* 1998) in chicks. Water soluble B-vitamins function as parts of coenzymes (Machlin 1984) and when present in deficient concentration in hens diet result in high embryonic mortality.

In ovo feeding technique is used to supply nutrients directly into the developing embryo for improvement in post-hatch growth (Ohta *et al*. 1999, Bhanja *et al*. 2004), immune responses (Konashi *et al.* 2000, Bhanja and Mandal 2005) and development of gastrointestinal tracts (Bhanja *et al.* 2008 a,b) providing major benefits to poultry growers. An effort was made to assess the effect of *in ovo* supplementation of vitamins at later stage of incubation and their effects on the hatchability, post-hatch performance, immunity and blood biochemical parameters in broiler chickens.

Present address: [1]Senior Research Fellow (genesakshat @gmail.com), [2]Principal Scientist (subratcari@gmail.com), Poultry Housing and Management, [4]Research Associate (dr.manishmehra @gmail.com),[5]Head, Avian Nutrition and Feed Technology. [3]Head (veena_biotech@rediffmail.com), Department of Biotechnology, Kumaon University, Bhimtal Campus, Bhimtal.

MATERIALS AND METHODS

In ovo treatments: Fertile eggs (600) were collected from the broiler breeder hens, weighed and distributed into 6 groups of 100 eggs each and set in a force draft incubator at temperature 37.5°C and relative humidity of 60%. First group acted as control (un-injected) and rest 5 groups were injected with vitamin A (105IU), vitamin B1 (18µg), vitamin B2 (36µg), vitamin B6 (35µg) or vitamin E (1.4IU) dissolved in 0.5 ml of sterile water on 14th day of incubation using a 24 gauge 25 mm needle at the broad end of egg following the method standardized by Bhanja *et al.* (2004). On 19th day of incubation the eggs were shifted to a hatcher and kept in pedigree hatching boxes.

Housing and management: On the day of hatch chicks were weighed, wing banded, sexed and transferred to 4-tier

electrically heated battery brooders, kept in a well-lit and ventilated open-sided house. Each treatment group had 6 replicates of 8 birds each and the chicks were reared up to 42 day of age and provided with a standard broiler ration. Food and water were available *ad lib.* All the chicks were vaccinated against Newcastle disease (ND, F1 strain) on 4th day post-hatch by occulo-nasal route.

Growth parameters: Bi-weekly body weight of individual birds and feed consumption by a group of eight birds in each pen were recorded and feed conversion ratio (FCR) was calculated, accordingly. Daily record of mortality, if any, for each treatment was maintained.

Immune responses

Humoral response: Sheep red blood cells (SRBC) suspended in Alsevar's solution were washed three times in isotonic phosphate buffer saline (PBS: pH 7.2) using centrifugation (700g) and adjusted to provide a 1% suspension (v/v) which was stored at 4°C prior to use. At 21 day of age 10 chicks from each treatment group were injected intravenously with 1 ml of SRBC suspension; 5 days later, blood sample (2ml) was obtained from the jugular vein of each chick and allowed to clot for serum collection. The antibody response to SRBC was determined using a standard haemagglutination assay (Siegel and Gross 1980, Van der Zijpp *et al.* 1983). The reciprocal of highest dilution showing clear agglutination was the end point of titer and the values were expressed as log 2.

ND vaccine response: Birds were vaccinated with ND on day 4 post-hatch and blood was collected on 14th day post vaccination. Serum was separated to study ND vaccine response by ELISA. Sample to positive ratio (S/P ratio) was calculated and then converted to titer value (log 10) using the formula, Log_{10} titer = 1.09 (Log_{10} S/P) + 3.36.

Blood bio-chemicals and organ weights: At 21st d post-hatch 2.0 ml blood was collected from 6 birds (equal sexes) of each treatment group and allowed to clot at room temperature. Serum was separated and subjected to blood biochemical analysis. Serum glucose, total protein and total cholesterol were estimated using standard kits. At 42nd day post-hatch, 6 birds (equal sexes) were killed and weight of the bursa, thymus and spleen were recorded and expressed as mg.bwt^{-1}. The weight of the digestive organs i.e empty gizzard plus proventriculus, intestine without chyme, liver and pancreas were recorded and expressed as g.bwt^{-1}.

Statistical analyses: Body weight of birds in each pen was analyzed sex-wise, whereas, FCR was calculated for each pen. Individual birds were the experimental unit for all immunological, blood biochemicals and organ study. Data were subjected to statistical analysis for ANOVA using standard procedure as described by Snedecor and Cochran (1980). Duncan Multiple Range Test (Duncan 1955) was used for verifying significant difference among treatment means.

RESULTS AND DISCUSSION

There was no difference in the weight of eggs used for *in ovo* injections or control group, but chick weight was significantly lower (P<0.05) in vitamin B6 injected eggs compared to un-injected control or other vitamin injected groups. *In ovo* vitamins injection did not affect hatchability and chick weight. Only vitamin B2 injected eggs had lower hatchability as compared to un-injected control group. *In ovo* feeding on 18th day of incubation had a negligible effect on hatchability (Uni and Ferket 2004). Moreover, vitamin B6 is known to reduce early embryonic death (Landauer 1967), this was quite evident in this study as good hatchability, comparable to un-injected control group was observed in vitamin B6 and E injected eggs (Table 1).

Body weight of male and female birds in different vitamins injected and control group are presented in Table 2. Not much variation was observed in the weight of male chicks at hatch, but eggs receiving vitamin B6 injection had significantly lower female chick weight. At 14th day post-hatch no variation was observed in the male chick weight but higher female body weight (20 to 32 g) was recorded in vitamin B1, B2 or E treatment in comparison to control group. The results corroborated with earlier studies on vitamin E injection (Bhanja *et al.* 2006) and mixture of vitamins containing vitamin B1, B2 and E (Bakyaraj *et al.* 2012) where increase in post-hatch body weight by 12 to 23g (5.3 to 13.3%) was seen in *in-ovo* fed birds. Similarly Uni *et al.* (2005) had also reported 5.1% higher body weight at 2 weeks of age and this advantage was sustained throughout 35 day of age in 18th day *in ovo* carbohydrate and protein fed birds. In our study vitamin B1 or B2 injected chicks had shown consistently higher body weight, and the difference reached 50 to 80g (3.6 to 5.8%) on 42nd day of marketable age in both male and female birds. This might be due to embryo's responses to vitamin B2 supplementation as it is involved in metabolism of nutrients (Couch *et al.* 1949, Robel and Christensen 1987).

Among vitamin injected groups, vitamin A or B1 injected

Table 1. Effect of *in ovo* vitamin injection on day-old chick weight and hatchability

Treatment	Egg wt (g)	Chick wt (g)	Hatchability (%)	Late hatch (%)
Control	68.03	46.01b	84.4	1.3
Vit A	69.03	45.70b	77.0	2.0
Vit B1	68.33	46.46b	77.0	2.0
Vit B2	68.45	45.9b	73.0	3.0
Vit B6	67.41	44.12a	84.0	2.0
Vit E	68.48	45.35ab	81.8	2.0
SEM	0.48	0.21	ND	ND
Significance	NS	P<0.05	ND	ND

ND, Not determined; [a–b] Means bearing different alphabets in a column differ significantly (P < 0.05).

Table 2. Biweekly body weight (g) of male and female broiler birds

Treatment	Day 0		Day 14		Day 28		Day 42	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	46.3	45.7b	312.8	271.0a	813.7	662.2	1524.6a	1232.3a
Vit A	46.6	44.7ab	312.7	289.0b	798.0	695.2	1513.3a	1262.7ab
Vit B1	46.6	46.2b	315.3	303.6b	840.9	700.0	1585.5b	1312.6c
Vit B2	45.9	45.8b	315.1	292.4b	834.6	705.6	1590.3b	1281.8bc
Vit B6	45.7	43.1a	306.2	270.6a	826.7	678.8	1519.6a	1227.1a
Vit E	45.1	45.5b	311.3	291.4b	834.2	664.4	1570.1ab	1262.5ab
SEM	0.2	0.3	2.1	2.5	5.6	5.6	8.3	6.7
Significance	NS	P<0.05	NS	P<0.01	NS	NS	P<0.01	P<0.01

[a–b] Means bearing different alphabets in a column differ significantly (P < 0.05).

Table 3. Feed conversion ratio (FCR) at different periods

FCR	0–14 day	14–28 day	28–42 day
Control	1.60ab	2.25	2.41
Vit A	1.54a	2.20	2.51
Vit B1	1.52a	2.21	2.40
Vit B2	1.60b	2.18	2.44
Vit B6	1.70b	2.09	2.54
Vit E	1.68b	2.20	2.49
SEM	0.01	0.02	0.018
Significance	P<0.01	NS	NS

[a–b] Means bearing different lowercases in a column differ significantly (P < 0.05).

chicks had better (P<0.01) FCR during 0 to 14 d of age than the vitamin B6 or E injected chicks, but did not vary from un-injected control group chicks. However, during 15 to 28 or 29 to 42 day of age there was no variation in the FCR of the birds among vitamin treated or un-injected control birds (Table 3). This finding is in line with the earlier studies conducted at our laboratory where either individual amino acids (Bhanja *et al*. 2012) or combination of vitamins (Bakyaraj *et al*. 2012) did not alter the FCR in broiler chickens. When comparison was made among vitamin injected chicks, those receiving vit A or vit B1 had better FCR than those treated with vit B2 or E during first 14 day of age. The benefit in vitamin B1 injected chicks could be through conversion thiamine (B1) to thiamine pyrophosphate in presence of ATP, which functions as a cofactor for several enzymes like pyruvate dehydrogenase and ex-ketoglutarate dehydrogenase and involved in conversion of glucose to energy, CO, and H_2 and O_2 (Buckle 1965).

Lymphoid organ weight and immune response of the birds receiving different vitamins injection have been presented in Table 4. No difference was observed in the weight of bursa in female but it was higher (P<0.01) in male birds of vitamin B1, B2 or E injected group. This finding is in line with the study of Marsh *et al*. (1986) who reported bursa as one of the most sensitive lymphoid organ in response to vitamin E and selenium affecting humoral immunity. Bakyaraj *et al.* (2012) had also reported higher bursa weight at hatch in fatty acid and vitamin-injected chicks. Spleen weight was higher in vitamin A injected male (P<0.01) and female (P<0.05) birds compared to un-injected control birds, while thymus weight (Table 4) was higher in vitamin A and B6 than control and Vitamin E treatment and also observed by Kurtoglu and Nizamloglu (1996) who reported increase in T-lymphocyte values in vitamins A and E treated chickens. Bursa is involved in the production of antibodies thus improving homoral immunity, whereas, thymus plays a role in celluar immune

Table 4. Immune response and lymphoid organ weight (mg.kg^{-1} body weight) at 42nd d post-hatch

Treatment	HA Titer (Log_2)	ND Titer (Log_{10})	Bursa		Spleen		Thymus	
			Male	Female	Male	Female	Male	Female
Control	8.8a	2.88	255.4a	257.7	210.0ab	187.7a	155.3a	166.8a
Vit A	8.8a	2.78	270.0a	232.5	285.7c	281.1b	189.0bc	191.2b
Vit B1	10.5b	2.86	356.9b	266.3	186.5a	192.4a	147.3a	155.2a
Vit B2	9.3ab	2.73	383.8b	277.8	180.9a	163.9a	162.8ab	154.8a
Vit B6	8.3a	2.73	288.1a	232.7	222.8b	192.4a	199.2c	179.5b
Vit E	9.1ab	3.13	361.6b	256.6	199.6ab	175.2a	142.6a	184.6b
SEM	0.2	0.06	13.0	7.1	9.1	12.2	6.1	3.6
Significance	P<0.05	NS	P<0.01	NS	P<0.01	P<0.05	P<0.01	P<0.01

[a–b] Means bearing different alphabets in a column differ significantly (P < 0.05).

Table 5. Digestive organ weight (g/kg^{-1} body weight) of broiler birds at 42nd day of age

Treatment	Gizard and proventriculus		Intestine		Liver		Pancreas	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	3.08	3.87	5.33a	5.48	2.53	2.33	0.35	0.31
Vit A	3.14	3.65	5.19a	5.93	2.47	2.72	0.36	0.43
Vit B1	2.93	3.4	5.9b	5.25	2.3	2.10	0.32	0.34
Vit B2	2.85	3.40	5.83b	6.09	2.37	2.29	0.32	0.31
Vit B6	3.35	3.73	5.22a	5.41	2.28	2.33	0.29	0.32
Vit E	3.02	3.35	5.2a	6.39	2.54	2.33	0.32	0.4
SEM	0.07	0.09	0.09	0.13	0.06	0.07	0.01	0.01
Significance	NS	NS	P<0.05	NS	NS	NS	NS	NS

[a–b] Means bearing different alphabets in a column differ significantly (P < 0.05).

responses. In the present study also the birds which had lager bursa had also larger thymus so expected to have better cellular immunity.

Antibody response against SRBC measured as HA titer (Log 2 value) was higher (P<0.05) in vitamin B1 treatment while no difference was observed in the titer values of other vitamin injected or un-injected control birds. Similarly, ND vaccine response measured as ND titer (Log 10 value) was apparently higher in vitamin E treatment but, not in others (Table 4). Bakyaraj *et al.* (2012) made similar observation, where *in ovo* injections of vitamins did not affect the humoral immunity status in broilers. Moreover, Bhanja *et al.* (2006) reported that a higher dose of vitamin E (0.75 IU) showed a lower anti-SRBC response than did a lower dose (0.5 IU). In the present study, a higher dose of vitamin E (1.4 IU) was injected so the response was not to the extent as expected. Lin and Chang (2006) also reported that vitamin E treatment groups had no significant difference in antibody titer responses to SRBC and NDV. However, Hossain *et al.* (1998) reported increase in antibody titer to killed-ND vaccine in chicks hatched from broiler breeders supplemented with vitamin E. Pyridoxine is functionally important as pyrodoxal phosphate in amino acid transformations and supporting protein synthesis needed for immune response. In our study male bursa weight and total protein level was higher in vitamin B6 treatment is line with the earlier study of Blalock *et al.* (1984) who observed that pyridoxine deficiency caused significant reduction in antibody concentrations to sheep red blood cells and relative concentrations of IgM and IgG during the peak and degradation phases of the primary response.

Weight of digestive organs of vitamin injected birds is presented in Table 5. No difference was observed in gizzard, liver or pancreas weight of male and female birds in comparison to un-injected control birds. The development of gut occurs throughout incubation and when embryo starts consuming amniotic fluid orally, intestinal weight increases from approximately 1% at 17 day of incubation to 3.5% at hatch. In our study *in ovo* feeding of vitamin B1 and B2 had higher (P<0.05) male intestinal weight, that is primary nutrient supply organ whose development would be certainly helpful in absorption of dietary nutrients and which correlates earlier studies of Coles *et al.* (2001) and Ferket and Uni (2002) who reported increase in jejunal glucose absorption and jejunum villi height in *in-ovo* peptide administration and saline containing carbohydrate at 18th day of incubation. In another studies Bhanja *et al.* (2008 a, b) had also reported increase in gastrointestinal tract weight in glucose injected chicks on the day of hatch and 10th day post-hatch.

The serum glucose, total protein and total cholesterol levels in vitamin and un-injected control birds were determined on 21st day of age and are presented in Table 6. Serum glucose level was significantly higher (P<0.01) in vitamin A, B2 or E injected birds in comparison to un-injected control birds. Serum protein level was higher (P<0.01) in vitamin B2, B6 or E treatment, while total cholesterol was higher (P<0.05) only in vitamin E injected birds. Higher serum glucose and protein level in vitamin B2 injected birds might be attributed to the ability of vitamin to breakdown the dietary carbohydrates, protein and fat by acting as co-factors for several enzymes. Kitakoshi *et al.* (2007) also reported higher blood sugar level but no difference in cholesterol level in vitamin B2 treated rats. Serum protein

Table 6. Effect of *in ovo* injection of vitamins on serum biochemical profiles

Treatment	Glucose mg/DL	Total protei ng/DL	Total cholesterol mg/DL
Control	131.7ab	3.21a	63.03a
Vit A	164.1c	3.29a	91.05ab
Vit B1	144.6b	3.35a	75.29a
Vit B2	169.5c	5.61c	70.81a
Vit B6	127.9a	4.42b	78.79a
Vit E	166.6c	5.97c	126.07b
SEM	3.4	0.21	5.79
Significance	P<0.01	P<0.01	P<0.05

[a–b]Means bearing different alphabets in a column differ significantly (P < 0.05).

level was significantly higher in vit E treated chicks. Ajakaiye *et al*. (2010) reported that vitamin E supplementation increased plasma protein concentration but decreased glucose and cholesterol concentrations in heat-stressed layer birds. Sahin *et al*. (2001) reported that serum glucose, uric acid, triglyceride, and cholesterol concentrations decreased significantly while protein and albumin concentrations increased when both dietary vitamin E and vitamin A were supplemented.

From the above study it may be concluded that vitamin B1 and B2 can improve growth while vitamin A, B1, B6 or E can modulate immunity in broiler chickens. There is a need for further research on how the birds response to the combinations of vitamins and whether it could be economically feasible for a routine practice in hatchery.

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