



## Effect of *In Ovo* Feeding of Different Levels of Vitamin B Complex on Hatchability, Production Performance, Carcass Characteristic, Blood Biochemical and Muscle Antioxidant of Broiler Chickens

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**T**HE aim of this study was to investigate the effect of *in ovo* feeding of different level of vitamin B complex (Bcx) on broilers production performance, carcass characteristic, blood biochemical and antioxidant status. During day 14 of incubation, 240 Cobb 500 eggs were purchased and evenly split among six groups according on the *in ovo* treatment. The control group was T1. In the meanwhile, groups T2, T3, T4, and T5 were given *in ovo* injections with 0.5 mL of distilled water and 100, 200, or 300 mg of vitamin Bcx per egg, respectively. The experimental groups did not differ in terms of chick weight upon hatching. In the meanwhile, there was no difference in feed consumption between the T3 and T5 groups and the control group in terms of final body weight and body weight increase from 1 to 5 weeks of age. The feed conversion ratio for animals aged 1 to 5 weeks did not vary significantly across groups. The relative weight of the bursa rose considerably in the T4 and T5 groups, but the relative weight of the spleen increased significantly in the T4 group compared to the control. As compared to the control group, *in ovo* vitamin Bcx treatment at 300 ng/egg significantly boosted serum albumin as well as muscle SOD and TAC activity. The current study found that *in ovo* injection of vitamin Bcx at a dose of 300 g/egg had positive effects on the relative weight of lymphoid organs and the antioxidant activity of muscles. Thus, more research is required to assess the effects of various *in ovo* vitamin B-group combinations on broiler performance for commercial adaption.

**Keywords:** *In ovo* feeding, vitamin B, production performance, carcass characteristic, Broiler Chickens

### Introduction

Embryonic development accounts for more than 33% of the total life cycle in modern commercial broiler lines. As a result, any disruptions during this time may affect the entire production cycle, resulting in significant losses for broiler producers [1]. The perinatal period, during which new muscle fibres are formed and matured, is crucial to the optimal development of the growing chick [2]. A complete nutrient deposit within the egg is critical for normal embryonic development and hatchability [3]. The continuous genetic selection for fast growing birds increases the energy and protein requirements for the developing embryo, resulting in an imbalance between growing embryonic nutritional requirements and the limited egg nutrient composition [4]. As a

result, a steady and sufficient supply of amino acids, vitamins, and minerals is required. *In ovo* feeding technology was advocated as an alternative to maximise productivity at early stages of the production cycle in order to achieve significant improvements in broiler production efficiency and profitability while also boosting immunity [1,2,5]. *In ovo* nutrient supplementation may assist embryos in overcoming the constraints associated with constant egg chemical composition, while also bracing chicks for the on-going intensive production. Vitamins and supplements such as probiotics, prebiotics, vaccines, and other nutraceuticals are currently used materials for *in ovo* feeding [1,4,6,7]. *In ovo* feeding is one of the most recent and effective methods of feeding

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embryos to improve performance by increasing the quality of hatched chicks, body weight at hatch, body weight gain post-hatch, and carcass yield, as well as improving nutrient metabolism, gut morphology, and immune response[8] Thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9), cobalamin (B12), choline, and nicotinic acid comprise the vitamin B complex. They function as coenzymes in a variety of intracellular reactions such as energy production, methyl donor generation, neurotransmitter synthesis, and immune functions[9] Vitamins B1, B2, and B3, for example, are required as cofactors in enzymatic reactions of anabolic pathways to produce energy in the Krebs cycle and electron transport chain during carbohydrate, lipid, and amino acid metabolism[10]. The release of glycerol and fatty acid molecules from triglyceride and phospholipid digestion is required for embryo completion, where fatty acids undergo  $\alpha$ -oxidation, producing acetyl-CoA, which enters the Krebs cycle and is oxidised to adenosine triphosphate (ATP) and carbon dioxide (CO<sub>2</sub>) [10]. During incubation, the heat production of the chicken embryo gradually increases from day 9 to 16, reaching a plateau phase from day 17 to 19. [11]. Furthermore, supplementation of vitamins B2, B5, B3, B9, and B12 at supra-nutritional levels improved the productive performance of broiler chickens raised in batteries and fed a corn and soybean-based diet [12]. Because B complex vitamins play numerous roles, their deficiency has a significant impact on the host's metabolism. Vitamin B12 injection during broiler egg incubation has been shown to improve hatchability as well as subsequent performance [13]. When vitamins B6 and B12 were injected *in ovo*, they reduced ethanol-induced oxidative stress in chicken embryos [14,15] reviewed several attempts at *in ovo* vitamin feeding. *In ovo* vitamin B1, B2, B6, and B12 injections improved hatchability and *in ovo* vitamin B1, B2, B6, and B12 injection improved hatchability and production performance, according to the researchers. Furthermore, *in ovo* vitamin B1, B2, and B9 injections boost immune system response [16]. However, the *in ovo* injection of a combination of B-group vitamins did not work. As a result, the current study was designed to look into the effect of *in ovo* vitamin B-group complex feeding at different levels on broiler chicken production performance, blood biochemical levels, and redox status.

## Material and Methods

### Ethical Approval

The authors confirm that the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received. The authors followed EU standards for the protection of animals used for scientific purposes. The study was conducted at the Agricultural Research and Production Station of National Research Centre, Al-Nubaria, Al-Beheira Governorate, Egypt.

### Experimental design

In total, 240 fertile Cobb 500 eggs were obtained from the same breeder flock. Eggs were weighed and eggs with a weight of  $60 \pm 1$  g were incubated in an automatic incubator at 37.5°C and 63% relative humidity (RH). At the 14<sup>th</sup> day of incubation, all eggs were examined by candling the egg to see its progress. The infertile or those containing early dead embryos were excluded. The fertile eggs were then distributed randomly into seven symmetric groups of 30 eggs each. On the 14<sup>th</sup> day of incubation, the injection site on eggs was cleaned with 70% ethanol alcohol and punctured with a hard, thin stylus before the vitamin B complex (Bcx) was injected into the yolk sac using an insulin syringe [17]. The injected vitamin Bcx was a combination of vitamins B1 (100 mg), B6 (50 mg) and B12 (0.5 mg) per mL. The experimental treatments were as follow; T1: without injection (control group), T2: eggs were injected with hole only (negative control), T3: eggs were injected with 0.5 mL of distilled water (positive control), T4: eggs were injected with 100 µg/egg vitamin Bcx, T5: eggs were injected with 200 µg/egg vitamin Bcx, and T6: eggs were injected with 300 µg/egg vitamin Bcx. All eggs were completed the rest of the incubation period at the same condition and were transferred from the setter tray to the hatcher basket at the 18<sup>th</sup> of incubation, with an incubation temperature of 37.5°C and 65 to 70% RH. At the end of the incubation period, the hatched chicks' were weighted (g) and hatchability rate were calculated as follow: Hatchability%= No. of chicks hatched/ No. of fertile egg incubated  $\times$  100.

### Birds Management

The hatched chicks in each treatment were distributed into 4 replicates, 10 chicks each. All chicks were reared under the same rearing condition for five weeks. The rearing temperature started at 32°C and the temperature was gradually decreased 3 degree each week until the end of the third week and then after that was constant at  $24 \pm 1$ °C. Diets were designed to meet the nutritional requirements according to NRC [18] and the Cobb 500TM management guidelines (Table 1). Diet was introduced in mash form and both feed and fresh water were available *ad libitum*.

### Production performance and carcass characteristics parameters

During the experimental period, the initial body weight was recorded per each group replica at the day of hatching and then weekly until the fifth week of old. Feed intake was recorded weekly. Then the body weight gain and feed conversion was calculated.

### Carcass characteristics

At the end of week five of age, 28 birds (4 birds per treatment, one from each replica) were chosen at

random and subjected to 8 hours of feed deprivation before slaughter, with the pre-slaughter weight recorded. Birds were slaughtered by cutting the jugular vein and leaving them for several minutes to bleed out completely. The slaughter weight was recorded, and the birds were then scalded and plucked. The carcass was opened after plucking, and edible and non-edible organs were removed to determine the dressed carcass weight. Carcass percent was determined as a percentage of live body weight. In addition, the liver, spleen, gizzard, heart, and bursa were all weighted and expressed as a percentage of live body weight. In addition, the length of the small intestine (duodenum-jejunum-ileum) was measured in centimetres. Dressing percentage (%) = [(Dressed carcass weight/Live body weight) 100].

#### Blood biochemical analysis and hormone testing

Blood samples were collected during exsanguination from five chicks per treatment in weatherman tubes from each group, centrifuged at 4000 rpm for 15 minutes, and serum samples were stored at -20°C until analysis. The serum concentrations of thyroxin (T4) and triiodothyronine (T3) were determined using the radioimmunoassay (RIA) technique [19]. Serum total protein and albumin were determined using a commercial kit and the guidelines of Doumas & Maume [20]. While globulin values were calculated by subtracting albumin values from total protein values. The albumin/globulin ratio was calculated, and Urea was measured using a commercial kit in accordance with the manufacturer's instructions [21].

Serum liver enzymes activity were also analyzed (AST) and alanine amino transaminases (ALT) using commercial kits (Linear Chemicals, Barcelona, Spain) according to the manufacturer procedure.

#### Antioxidant activity

Muscle samples were obtained after slaughter (4 birds per treatment one from each replica) and were immediately put in liquid nitrogen until further

analysis. Liver samples were thawed and manually homogenized in cold phosphate buffer. After centrifugation the supernatant was removed. The activity of superoxide dismutase (SOD), catalase and the total antioxidant capacity (TAC) were measured in the supernatant using the commercial assay kits from Nanjing Jiancheng Biochemistry Reagent Co (NJBC, Nanjing, Jiangsu, China; according to the instructions of the manufacturer. Each sample was assayed in duplicate.

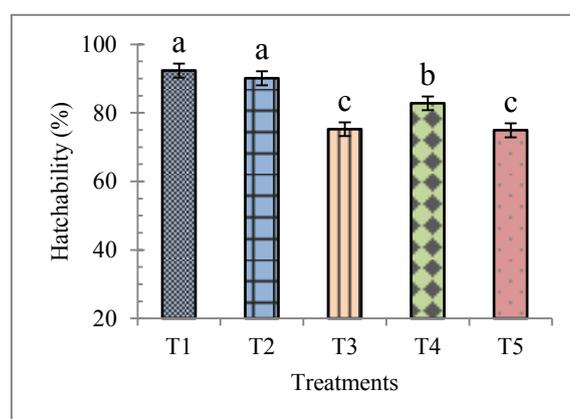
#### Statistical analysis

Data of the experiment were analyzed statistically using the one-way analysis of variance (ANOVA) with the SPSS [22]. Differences among means were determined using the Duncan's multiple range test [23].

## Results and Discussion

### Hatchability and production performance

The hatchability of eggs fed *in ovo* with various levels of vitamin B complex is shown in Fig (1). Data showed that groups injected with vitamin B complex had a significantly lower hatchability percentage than the control and sham groups. When compared to the control group, the reduction was more pronounced for T3 and T5, with 19% reductions, followed by T4 with 10% reductions. This finding was similar to that of Akshat *et al.* [24], who discovered that eggs injected with vitamin B2 had lower hatchability than un-injected control eggs. Also, adding vitamin B6 in the early embryonic stage resulted in embryonic growth retardation, resulting in death and, eventually, poor hatchability. On the other hand, Ibrahim *et al.* [25], discovered that *in ovo* feeding on the 18th day of incubation had no effect on hatchability. However, Uni & Ferket [26] discovered that *in ovo* feeding on the 18<sup>th</sup> day of incubation had no effect on hatchability. This finding could be explained by a significant decrease in brain Homocysteine (HoCys) on the 15<sup>th</sup> day of embryonic development in eggs injected with B vitamins [27].



**Fig. 1. Hatchability % of eggs subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Column with different superscript letter significantly differ ( $p < 0.05$ ) T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

The production performance parameters of broiler chickens subjected to *in ovo* vitamin B complex feeding at various levels are presented in Table (1). Hatching weight and body weight (BW) did not differ between groups at week 3 of age. Meanwhile, BW decreased significantly in the group subjected to *in ovo* feeding with vitamin B complex T3 and T5 at the fifth week of the experiment when compared to the control and sham groups. Bhanja *et al.* [28] obtained comparable results, reporting that there were no significant differences in body weight between vitamin B injection groups, sham control groups, and un injected birds at 14 days old. On the other hand, Babak *et al.* [29] discovered that chicks born after *in ovo* injection of eggs with vitamin B1 or B2 had higher body weight, ranging from 50 to 80g (3.6 to 5.8%) on the 42day of marketable age in both male and female birds. Furthermore, during the first three weeks of life, Body weight gain (BWG) did not differ between experimental groups. However, BWG varied

significantly between the fourth and fifth weeks of life, as well as throughout the experiment. The T5 group had the lowest BWG, which was significantly lower than the control and T4 groups. Furthermore, when compared to the control and sham groups, BWG showed a significant reduction in T4 and T5 during the experimental period. Our results agreed with those of Bhanja *et al.* [28], who found no significant effect on LBW at 14 days of age after injecting broiler eggs with vitamin B6 (100 g/egg) dissolved in 0.5 ml of sterile water and found no statistically significant effect on LBW at 14 days of age. In contrast, Akshat *et al.* [24] discovered that injecting vitamin B12 into incubated eggs on the first day of incubation resulted in greater live body weight and body weight gain of hatched ducklings during the growing period (0-8 weeks) than the control group (non-injected) During weeks 4 and 5, feed intake of T5 decreased significantly more than T4, but vitamin B had no effect on feed intake in any group.

**TABLE 1. Production performance of broiler chickens subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Item	T1	T2	T3	T4	T5	SEM	P-value
<b>Body weight, g</b>							
1 <sup>st</sup> Day	37.58	36.42	36.00	34.75	36.00	0.40	0.275
3 <sup>rd</sup> Week	647.95	631.26	607.86	550.75	588.63	19.04	0.590
5 <sup>th</sup> Week	1628.85 <sup>a</sup>	1578.83 <sup>a</sup>	1446.98 <sup>bc</sup>	1543.69 <sup>ab</sup>	1404.53 <sup>c</sup>	25.29	0.003
<b>Body weight gain, g</b>							
1 – 3 Weeks	610.37	594.85	571.86	516.00	552.63	18.96	0.614
4 – 5 Weeks	980.90 <sup>a</sup>	947.57 <sup>ab</sup>	839.12 <sup>ab</sup>	992.94 <sup>a</sup>	782.57 <sup>b</sup>	29.35	0.055
1 – 5 Weeks	1591.27 <sup>a</sup>	1542.42 <sup>a</sup>	1410.9 <sup>bc</sup>	1508.94 <sup>ab</sup>	1368.53 <sup>c</sup>	25.18	0.003
<b>Feed intake, kg</b>							
1 – 3 Weeks	1.15	1.08	1.12	1.04	1.11	0.03	0.906
4 – 5 Weeks	2.35 <sup>ab</sup>	2.40 <sup>ab</sup>	2.18 <sup>ab</sup>	2.60 <sup>a</sup>	2.09 <sup>b</sup>	0.07	0.159
1 – 5 Weeks	3.50	3.48	3.29	3.63	3.21	0.08	0.566
<b>Feed conversion ratio</b>							
1 – 3 Weeks	1.88 <sup>bc</sup>	1.82 <sup>c</sup>	1.95 <sup>ab</sup>	2.01 <sup>a</sup>	2.02 <sup>a</sup>	0.02	0.001
4 – 5 Weeks	2.40 <sup>c</sup>	2.53 <sup>b</sup>	2.60 <sup>ab</sup>	2.62 <sup>ab</sup>	2.67 <sup>a</sup>	0.03	0.003
1 – 5 Weeks	2.20	2.26	2.33	2.41	2.34	0.05	0.741

Means with different superscript letter in the same column significantly differ ( $P < 0.05$ ).

T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

The results of current study were supported by some researchers [30, 31] who discovered no significant differences in average daily feed intake between the control and folic acid-injected treatments. However, some authors [32,29] found that *in-ovo* chickens injected with folic acid (vit. B)

had significantly higher feed intake than the control. When compared to the control group, the feed conversion ratio (FCR) was lower in T5 from 1 to 3 weeks and 4 to 5 weeks of age, however, no significant difference between groups was observed throughout the entire experimental period. The

findings of the current study agreed with those of Amal *et al.* [31] who discovered that *in-ovo* injection had no effect on FCR) in any of the age groups investigated. However, when compared to the control treatment, FCR in broilers injected with 120 g folic acid in albumen on day 7 of incubation was significantly improved on 0-42 days, according to Nouri *et al.* [32]. Also, [29] discovered that *in ovo* vitamin B12 feeding improved feed conversion ratio ( $p < 0.05$ ) at 5 weeks of age. This result could be attributed to embryo responses to vitamin B2 supplementation, as it is involved in nutrient metabolism.

### Carcass characteristics

Relative percentage of different carcass parts is presented in Table (2). There were no significant

differences in dressing, liver, gizzard percentages, or intestine length between groups. The current study's findings were consistent with those of Babak *et al.* [29], who discovered no discernible effect of vitamin B12 supplementation on slaughter weight, carcass weight, and dressing percentage, as well as Nouri *et al.* [32], who discovered no significant differences in carcass characteristics of broilers that were *in ovo* injected with folic acid (40, 80, and 120 g) compared to the control group at the age of 42 days. In contrast, NRC [18] found that *in ovo* folic acid injection significantly increased broiler carcass meat yield.

**TABLE 2. Carcass characteristics of broiler chickens subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Treatment	Dressing %	Heart %	Liver %	Gizzard %	Bursa %	Spleen %	Intestine length, cm
T1	73.36	0.50 <sup>b</sup>	3.00	1.45	0.078 <sup>b</sup>	0.21 <sup>b</sup>	158.25
T2	73.72	0.69 <sup>a</sup>	3.46	1.28	0.091 <sup>a</sup>	0.22 <sup>b</sup>	181.25
T3	74.39	0.48 <sup>b</sup>	2.76	1.20	0.079 <sup>b</sup>	0.22 <sup>b</sup>	177.50
T4	74.47	0.62 <sup>ab</sup>	3.25	1.15	0.089 <sup>a</sup>	0.34 <sup>a</sup>	163.75
T5	74.23	0.64 <sup>ab</sup>	3.33	1.40	0.086 <sup>a</sup>	0.17 <sup>b</sup>	172.50
SEM	0.48	0.03	0.13	0.05	0.001	0.02	5.06
P-value	0.955	0.048	0.474	0.218	<0.0001	0.004	0.629

Means with different superscript letter in the same column significantly differ ( $P < 0.05$ ).

T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

However, in this study, the sham group's heart% was significantly higher than the control and T3 groups. Furthermore, bursa% increased significantly in the sham, T4 and T5 groups when compared to the control group. Furthermore, spleen percentage increased significantly in the T4 group compared to the other treatment groups. This result agreed with El-Kholy *et al.* [33], who reported that greater weights of immune organs generally represent associations with this showed stronger immune function compared to other treatments, but not with Amal *et al.* [31], who discovered that higher weight of the bursa gland could be used as an immune organ, reported that relative weights of some immune organs (bursa gland) could be used to assess immune status. The percentage of spleen, bursa, and thymus, which are all lymphoid organs, was not significantly impacted by *in ovo* injection treatments

### Blood metabolites and thyroid hormones level

Blood biochemical parameters of broiler exposed to *in ovo* feeding with various Bcx vitamin levels are presented in Table (3). When compared to the control group, serum total protein and albumin levels increased in all treated groups. Meanwhile, serum urea levels and the activities of the AST and ALT enzymes did not differ between groups. Thyroid hormone levels, on the other hand, were measured in plasma. This result was in agreement with those of Babak *et al.* [29], They found that broilers' TP and albumin levels increased significantly ( $P < 0.05$ ) when vitamin B12 was administered into eggs *in ovo* on day 42 after hatching. The blood protein level was higher ( $P < 0.05$ ) following vitamin B2 and B6 treatment, according to Akshat *et al.* [24], who reported the same outcome.

**TABLE 3. Blood metabolites of broiler chickens subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Treatment	TP (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio	Urea (mg/dL)	AST (U/L)	ALT (U/L)	T <sub>3</sub> (ng/mL)	T <sub>4</sub> (ng/mL)
T1	2.28 <sup>b</sup>	0.94 <sup>b</sup>	1.11 <sup>b</sup>	1.09	2.64	203.84	13.82	6.83	16.67 <sup>b</sup>
T2	2.98 <sup>a</sup>	1.35 <sup>a</sup>	1.63 <sup>a</sup>	0.83	2.57	197.62	13.97	7.80	19.73 <sup>ab</sup>
T3	3.14 <sup>a</sup>	1.63 <sup>a</sup>	1.51 <sup>ab</sup>	1.09	2.57	209.02	13.50	7.30	19.87 <sup>ab</sup>
T4	3.03 <sup>a</sup>	1.41 <sup>a</sup>	1.62 <sup>a</sup>	0.91	2.53	199.16	13.84	7.17	20.73 <sup>ab</sup>
T5	3.08 <sup>a</sup>	1.46 <sup>a</sup>	1.58 <sup>a</sup>	0.94	2.55	198.75	13.95	7.57	21.03 <sup>a</sup>
SEM	0.11	0.07	0.07	0.06	0.03	2.14	0.11	0.15	0.62
<i>P</i> -value	0.040	0.011	0.129	0.591	0.827	0.464	0.724	0.298	0.168

Means with different superscript letter in the same column significantly differ ( $P < 0.05$ ).

T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

Similar results were observed by El-Kholy *et al.* [33], who found that *in ovo* injections of 150 g/egg vitamin B6 and 20 g/egg vitamin B12 increased plasma total protein, plasma albumin, and globulin significantly ( $P < 0.05$ ) in comparison to both control groups.

However, the A/G ratio was unaffected by any of the *in ovo* injection treatments ( $P > 0.05$ ). Since vitamin B functions as a cofactor for many enzymes, it can help break down protein, carbs, and fat, which may explain why the blood protein level was higher in the vitamin B-injected birds. In the current study, plasma T3 levels did not differ between groups. However, as compared to the control group, T4 levels in the T5 group dramatically rose. The release of T4 from the thyroid gland into the blood

circulation during hatching was shown to be considerably greater in the group treated with riboflavin at a dosage of 600 g/egg compared to the control group by Trzeciak *et al.* [34]. Nevertheless, vitamin injection increased plasma T3 and T4 levels [33]. These results were consistent with those of El-sayed *et al.* [35], who found that quails hatching from eggs injected with 120 g of B6 per egg had significantly ( $P < 0.05$ ) higher blood T3 concentrations. It is possible to conclude that the observed changes in T4 levels can be attributed to hypothalamic-pituitary-thyroid (HPT) axis activity under the influence of vitamin B, as well as changes in the metabolism of this hormone in peripheral tissues such as the liver (Table 4).

**TABLE 4. Serum thyroid hormones levels of broiler chickens subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Treatment	T3 (ng/mL)	T4 (ng/mL)	T4/T3 ratio
T1	6.83	16.67 <sup>b</sup>	2.43 <sup>c</sup>
T2	7.80	19.73 <sup>ab</sup>	2.53 <sup>bc</sup>
T3	7.30	19.87 <sup>ab</sup>	2.72 <sup>ab</sup>
T4	7.17	20.73 <sup>ab</sup>	2.89 <sup>a</sup>
T5	7.57	21.03 <sup>a</sup>	2.78 <sup>ab</sup>
SEM	0.15	0.62	0.06
<i>P</i> -value	0.298	0.168	0.019

Means with different superscript letter in the same column significantly differ ( $P < 0.05$ ).

T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

### Antioxidant activity

The SOD and catalase enzyme activity and the total antioxidant capacity in muscle of broiler chicken subjected to *in ovo* feeding with different levels of vitamin Bcx are presented in Table (5). SOD enzyme activity increased significantly in the T5 group compared to the control. Furthermore, when compared to the control and other treated groups, the T5 group's total antioxidant capacity increased

significantly. Muscle catalase activity, on the other hand, did not differ between experimental groups. This result is similar to other studies found that adding high concentration of riboflavin could increase antioxidant parameters like SOD, malondialdehyde and glutathione peroxidase [36]. This finding is consistent with other studies that found that increasing antioxidant parameters such as SOD, malondialdehyde, and glutathione peroxidase

by using a high concentration of riboflavin [37]. In addition, Gouda *et al.*[38] discovered that feeding folic acid at a rate of 1.5 mg/kg for 35 days improved performance. And also, discovered that feeding 1.5 mg/kg folic acid for 35 days improved broiler antioxidant status by increasing heat shock protein 70 (HSP70), total antioxidant capacity (TAC), and superoxide dismutase enzyme (SOD) activity under

heat stress. However, increased doses of vitamin B did not increase serum SOD activity in other studies. Based on these findings, it can be concluded that adding higher concentrations of vitamin B cause a significant increase in serum concentrations of TAC and SOD. This improvement may be due to the homocysteine-lowering mechanism and its ability to reduce oxidative stress.

**TABLE 5. Antioxidant activity of broiler chickens subjected to *in ovo* feeding with different levels of vitamin B complex (Bcx).**

Treatment	Muscle SOD (U/g)	Muscle TAC (mML)	Muscle catalase (U/g)	Plasma catalase (U/L)
T1	0.77 <sup>b</sup>	1.01 <sup>b</sup>	0.65	0.72
T2	0.85 <sup>ab</sup>	1.20 <sup>b</sup>	0.68	0.89
T3	0.80 <sup>ab</sup>	1.17 <sup>b</sup>	0.65	0.83
T4	0.95 <sup>ab</sup>	1.26 <sup>b</sup>	0.66	0.87
T5	0.96 <sup>a</sup>	1.58 <sup>a</sup>	0.68	0.88
SEM	0.03	0.06	0.01	0.03
P-value	0.106	0.013	0.742	0.421

Means with different superscript letter in the same column significantly differ ( $P < 0.05$ ).

T1: control group, T2: eggs were injected with 0.5 mL distilled water, T3: eggs were injected with 100 mg/egg vitamin Bcx, T4: eggs were injected with 200 mg/egg vitamin Bcx, and T5: eggs were injected with 300 mg/egg vitamin Bcx.

## Conclusion

In conclusion, we were unable to find a comparable paper to compare the results of a limited study on *in ovo* feeding of a combination of vitamin B on broiler performance. According to the findings of this study, *in ovo* feeding of a combination of vitamins B1, B2, and B12 had a negative effect on hatchability while improving broiler chicken subsequent production performance. The significant increase in bursa and spleen relative weight (primary and secondary lymphoid organs) in the vitamin Bcx *in ovo* injected group suggests an immunomodulation response role. With vitamin Bcx *in ovo* injection, muscle antioxidant activity increased significantly, implying improved meat quality and increased shelf life. It can be deduced that *in ovo* vitamin B1, B2, and B12 injections can be used to improve with no effect on carcass or vital organ activities. Further investigations on the effect of vitamin Bcx *in ovo* feeding on different immune response are needed.

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## Conflict of interest

The authors declare that they have no conflict of interest

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## تأثير تغذية البويضات بمستويات مختلفة من فيتامين ب المركب على قابلية الفقس ، أداء الإنتاج ، خصائص الذبيحة ، التحايل الكيمياءى للدم ومضادات الأوكسدة فى العضلات لدجاج اللحم

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دراسة تأثير تغذية البويضات بمستويات مختلفة من فيتامين ب المركب (Bcx) على أداء إنتاج دجاج التسمين وخصائص الذبيحة والحالة الكيميائية الحيوية للدم ومضادات الأوكسدة. خلال اليوم 14 من الحضنة ، تم شراء 240 كوب 500 بيضة وتم تقسيمها بالتساوي بين ست مجموعات وفقاً لمعاملة البيض. كانت المجموعة الضابطة T1 في غضون ذلك ، تم إعطاء المجموعات T2 و T3 و T4 و T5 في حقن البويضات مع 0.5 مل من الماء المقطر و 100 أو 200 أو 300 جم من فيتامين Bcx لكل بيضة ، على التوالي. لم تختلف المجموعات التجريبية من حيث وزن الكتاكيت عند الفقس. في غضون ذلك ، لم يكن هناك فرق في استهلاك العلف بين المجموعتين T3 و T5 ومجموعة التحكم من حيث زيادة وزن الجسم النهائي ووزن الجسم من 1 إلى 5 أسابيع من العمر. لم تختلف نسبة تحويل العلف للحيوانات التي تتراوح أعمارها بين 1 إلى 5 أسابيع بشكل كبير عبر المجموعات ، فقد ارتفع الوزن النسبي للبرسا بشكل كبير في مجموعتي T4 و T5 ، لكن الوزن النسبي للطحال زاد بشكل ملحوظ في مجموعة T4 مقارنة بمجموعة التحكم. بالمقارنة مع المجموعة الضابطة ، في علاج فيتامين Bcx عند 300 نانوغرام / بيضة عزز بشكل ملحوظ ألبومين المصل وكذلك نشاط العضلات SOD و TAC. وجدت الدراسة الحالية أن حقن البويضات لفيتامين Bcx بجرعة 300 جم / بيضة كان له آثار إيجابية على الوزن النسبي للأعضاء اللمفاوية والنشاط المضاد للأوكسدة للعضلات. وبالتالي ، هناك حاجة إلى مزيد من البحث لتقييم آثار مجموعات فيتامين ب المختلفة في البيض على أداء دجاج التسمين من أجل عملية التأقلم التجاري.

**الكلمات المفتاحية:** تغذية البويضات ، فيتامين ب ، أداء الإنتاج ، خصائص الذبيحة ، دجاج التسمين .