

# Effects of boron supplementation of adequate and inadequate vitamin D<sub>3</sub>-containing diet on performance and serum biochemical characters of broiler chickens

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## SUMMARY

In this study, supplementation of two levels (5 and 25 parts per million; ppm) of boron into broiler diets including 125 IU kg<sup>-1</sup> (inadequate) and 2000 IU kg<sup>-1</sup> (adequate) vitamin D<sub>3</sub> as investigated. The effects of supplementation on performance and biochemical characters (Ca, P, Mg, glucose and AP) of broilers from 1 to 45 days of age were evaluated. Boron provided significant increases in performances of chicks fed both adequate and inadequate vitamin D<sub>3</sub>-containing diets. The improvements in the inadequate vitamin D<sub>3</sub>-containing group were higher than that of adequate vitamin D<sub>3</sub>-containing group. The boron addition had a positive effect on Ca, P and alkaline phosphatase levels of chicks. Boron might be regarded as beneficial in inadequate vitamin D<sub>3</sub>-containing broiler feed. © 2001 Harcourt Publishers Ltd

BORON (B), is a dark brown non-metal that is found in nature as borax, colemanite, boronatrocalcite, and boracite. It acts as a Lewis acid, accepting hydroxyl ions, and thus leaving an excess of protons. Boron complexes with organic compounds containing hydroxyl groups, and those with more than two hydroxyl react more strongly. Thus, B is capable of interacting with substances of biological interest, including polysaccharides, pyridoxine, riboflavin, dehydroascorbic acid, and pyridine nucleotides (Naghii and Samman, 1993). The average B content (parts per million; ppm) in dry weight, in different food groups is as follows: cereals 0.92, meat 0.16, fish 0.36; dairy products 1.1 and vegetable foods 13 (Nielsen, 1992). Boron is distributed throughout the tissues and organs of animals at concentrations mostly between 0.05 to 0.06 ppm fresh weight and several times these levels in the bones (Underwood, 1977).

Boron has been known to be essential for higher plants since the 1920's, but only recently has a possible role in animal and human nutrition been suggested. In a recent review, Loomis and Durst (1992) listed the roles of B in plants that have been postulated over the years. These roles involve sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, membrane functions, and DNA synthesis. Boron deficiency occurs in some plants when vegetative dry matter contains less than 15 ppm B (Hunt and Nielsen 1981).

Several studies have indicated that B is an important mineral for body weight, feed consumption, reduced

mortality rate, normal cartilage and bone formation in broilers. Rossi et al (1993) investigated the effects of supplementing B in a maize-soybean diet on body weights of broilers. Generally, B increased body weights of both male and female broilers. Hunt and Nielsen (1981) supplemented a purified diet containing various levels of B and either adequate or inadequate amounts of vitamin D<sub>3</sub>. They found a positive relationship between B and vitamin D<sub>3</sub>. Boron supplementation improved chick body weight when the vitamin D<sub>3</sub> content of the diet was adequate (2500 IU kg<sup>-1</sup>) and vitamin D<sub>3</sub> was inadequate (125 IU kg<sup>-1</sup>). The same researches (Hunt and Nielsen 1987) also reported that 4 weeks of B deprivation resulted in chick growth depression, elevated plasma glucose and increased brain weight to body weight ratio. Elliot and Edwards (1990) used a factorial arrangement of treatments involving the addition of calcium, vitamin D<sub>3</sub> and B to purified diets for broilers. They reported that B supplementation tended to increase bone ash and that there was a significant interaction between B and vitamin D<sub>3</sub> on body weight gain.

Boron has been examined as a possible nutritional factor in calcium (Ca) metabolism and utilisation and, thus, as a factor in the development and maintenance of normal bone (Nielsen 1992). Bone-breaking strength and bone ash are often used as criteria for assessing the values of various dietary supplements, cage designs and animal densities for preventing bone breakage. For this reason, The National Research Council (NRC 1984) suggested that trace mineral supplements to chemically defined diets should contain at least 2 ppm B, although the B requirement for the different categories of poultry has not been determined (Hunt 1989, Nielsen and Shuler 1992, Rossi et al 1993).

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It was suggested that B supplementation in chicks tends to alleviate signs of vitamin D<sub>3</sub> deficiency such as depressed growth rate and increased plasma alkaline phosphatase (AP) (Hunt and Nielsen 1981). Also, Hunt et al (1983), in a series of experiments with day-old chicks fed for 30 days, indicated a relationship between B and other nutrients (Ca, Mg, and vitamin D<sub>3</sub>). Signs of B deficiency may be related to the level of vitamin D<sub>3</sub>, Mg and possibly other nutrients in the diet. Indeed, comparison of the findings on growth from the boron-calcium (B-Ca) and boron-magnesium (B-Mg) studies suggested that the relationship between B-Mg was stronger than that between Ca or P and B. Hunt et al (1994) reported that B acts by at least three different mechanisms. First, B compensates for perturbations in energy substrate utilisation induced by vitamin D<sub>3</sub> deficiency. Second, B enhances macro mineral content in normal bone. Third, dietary B independently of vitamin D<sub>3</sub>, enhances some indices of growth cartilage formation. Nielsen et al (1988) indicated, this function of B related to its parathormone regulating action. Therefore B is needed by the parathyroid and has been shown to prevent loss of Ca and bone demineralisation in post-menopausal women.

The purpose of this study was to evaluate the effects of two levels of B supplementation in broiler chicks fed adequate (2000 IU kg<sup>-1</sup>) or inadequate (250 IU kg<sup>-1</sup>) vitamin D<sub>3</sub> by observing their effects on performance and various serum biochemical components, in chicks from 1 to 45 days of age.

## MATERIALS AND METHODS

### Chicks and experimental design

In this experiment, 540 1-day-old, unvaccinated broiler chicks (*Avian*) were used. Chickens were weighted individually and divided into six groups as shown in Table 1. To limit the position differences, groups were divided into six subgroups, consisting of 15 chicks each. The chicks were placed in houses heated and lit by a fluorescent source. Feed and drinking water were available *ad libitum*. The experimental period lasted to the 45th day.

### Diet

In the trial, chickens received basal diet (as shown Table 2). The vitamin premix, B, and vitamin D<sub>3</sub> for each group (Table 1) were weighted individually and

**TABLE 1: Boron and vitamin D<sub>3</sub> supplementation to diets used in groups.**

Group	Boron (B) mg kg <sup>-1</sup> food	Vitamin D <sub>3</sub> IU kg <sup>-1</sup> food
1	0	250
2	0	2000
3	5	250
4	5	2000
5	25	250
6	25	2000

**TABLE 2: Compositions of diets used in the experiments.**

Source	Days	
	1-28	28-45
Maize	55.30	47.15
Wheat	-	14.80
Soybean meal	23.00	12.05
Sunflower meal	2.50	-
Fish meal	6.00	3.00
Bone meal	-	2.00
Full Fat	7.80	15.00
Vegetable oil	2.43	-
Mixed oil	-	3.50
Limestone	1.70	0.70
DCP	0.60	0.57
Salt	0.15	0.27
Mineral premix*	0.10	0.20
Vitamin premix**	0.25	0.25
Antioxidant	-	0.10
Coccidiostat	-	0.10
Methionine	0.17	0.23
Lysine	-	0.08
Chemical analysis results		
ME***, kcal	3175.24	3300
Crude protein, per cent	23.00	19.00
Dry matter, per cent	89.13	89.02
Crude ash, per cent	5.61	5.11
Crude cellulose, per cent	3.60	3.06
Ether extract, per cent	6.96	8.89

\*Mineral premix: In each kg; Mn 80.000 mg; Fe 35.000 mg; Zn 50.000 mg; Cu 5.000 mg; I 2.000 mg; Co 400 mg; Se 150 mg;

\*\*Vitamin premix: In each 2.5 kg; Vit A 12.000.000 IU; Vit D<sub>3</sub> 2.000.000 IU; Vit E 30.000 mg; Vit K<sub>3</sub> 3.000 mg; Vit B<sub>1</sub> 3.000 mg; Vit B<sub>2</sub> 6.000 mg; Vit B<sub>6</sub> 5.000 mg; Vit B<sub>12</sub> 15 mg; Niacin 25.000 mg; Biotin 40 mg; Carotenoid 8.000 mg; Folic acid 1.000 mg; Cholin chloride 300.000 mg; Vit C 50.000 mg; \*\*\*Calculated.

supplemented to diets in homogenous form. Orthoboric acid was used as the B source since it is a common inorganic form of B of high purity (99.995 per cent) and absorbed well from the gastrointestinal tract (Pfeiffer and Jenney 1950).

Crude nutrients in feed were analysed by the methods of AOAC (1984)

### Performance and biochemical measurements

Body weight (BW) and food consumption (FC) of chickens were determined on 1st, 14th, 28th, 35th and 45th days of age. Serum Ca, Mg, P, glucose and AP levels were measured with spectrophotometer (Shimadzu, UV 2100) using commercial kits (Pointe Scientific Inc., Lincoln Park, Michigan, USA). Blood samples were taken from 12 chicks in each group by cardiac puncture on the 45th day.

### Statistical analysis

Data belonging to performance such as BW, FC and food conversion ratio (FCR) and biochemical characters (Ca, P, Mg, glucose and AP) were measured individually and analysed using Duncan's multiple range test (SPSS 1998).

## RESULTS

The addition of 5 and 25 mg kg<sup>-1</sup> B to the diets containing inadequate vitamin D<sub>3</sub> (groups 3 and 5) caused a significant increase in BW values by days 14 and 28 (Table 3) compared to the inadequate D<sub>3</sub> diet not supplemented with B (group 1). The highest BW value on the 28th day was in group 6 which were fed the highest values of B and D<sub>3</sub>; it was significantly greater than group 1 (P < 0.001). The differences in BW on day 45 were not significantly different in the groups.

The food consumption (FC) values (Table 4) were highest in days 1–35 in the group on the lowest D<sub>3</sub> and the lowest boron but in the period between days 1 and 45, there was no significant difference among groups.

Table 5 shows that food conversion (FCR) was best with high boron and high D<sub>3</sub> from day 1–14 (Table 5) and together with high boron and high D<sub>3</sub> from day 1–28. However, in the day 1–45 period there was no significant difference in any of the groups.

Table 6 shows that compared with serum calcium in the group on low D<sub>3</sub> and no boron (group 1) that calcium was significantly reduced by supplementing the diet with 25 mg kg<sup>-1</sup> of B (groups 5 and 6). This was not seen in diets with the higher value of D<sub>3</sub> (groups 2 and 4). The highest concentration of phosphorus was obtained with the highest level of boron and D<sub>3</sub> (group 6) and similar but lesser values were obtained with low boron and high D<sub>3</sub> (group 4) and high boron and low D<sub>3</sub> (group 5).

There was significant reduction in AP by single or dual supplementation of diet with B and D<sub>3</sub> in groups 2 to 6 inclusive compared with group 1.

No significant effects were noted in glucose and Mg values.

## DISCUSSION

Boron supplementation to broiler diets has become a concern of the broiler industry because of the importance of B to BW, FC and Ca metabolism and bone formation (Wilson and Ruzsler, 1997, Hunt 1989). Hunt and Nielsen (1981) found a positive relationship between B and D<sub>3</sub> and reported that BW was improved by supplementation of the diet with B when the D<sub>3</sub> content was either inadequate (125 IU kg<sup>-1</sup>) or adequate (2500 IU kg<sup>-1</sup>). Rossi et al (1993) reported similar findings with diet containing 2200 IU D<sub>3</sub> supplemented with 5 mg kg<sup>-1</sup> of B. In contrast, Elliot and Edwards (1990) found no differences in BW when the diet was supplemented with 20, 40 and 80 mg kg<sup>-1</sup> of B. In our study we observed the effect of supplementing a diet with a low (5 mg kg<sup>-1</sup>) and high (25 mg kg<sup>-1</sup>) levels of B with inadequate (250 IU) and adequate (2000 IU) levels of D<sub>3</sub>. As shown in the results even low level supplementation with B greatly improved BW to about 28 days of age and the best BW to this age was obtained with supplementing with high B and high D<sub>3</sub>.

Rossi et al (1993) reported that there were no significant differences in FC in a 21-day period with an

**TABLE 3: Effect of Boron (B) and vitamin D<sub>3</sub> on body weight (bw) for broiler chicks\***

Group number	Treatment				Body weight (g)					
	Bmg kg <sup>-1</sup>		Vit D <sub>3</sub> IU kg <sup>-1</sup>		1**	14	28	35	45	
	5	25	250	2000						
1	-	-	+	-	38.12 ± 0.43	363.27 ± 7.43 <sup>b</sup>	988.72 ± 11.16 <sup>c</sup>	1360.66 ± 18.45	2106.54 ± 26.62	
2	-	-	-	+	38.41 ± 0.33	357.40 ± 6.75 <sup>b</sup>	994.11 ± 21.09 <sup>c</sup>	1381.37 ± 29.71	2119.51 ± 33.86	
3	+	-	+	-	37.89 ± 0.45	399.02 ± 4.69 <sup>a</sup>	1064.57 ± 30.55 <sup>ab</sup>	1425.16 ± 46.76	2121.17 ± 70.33	
4	+	-	-	+	38.94 ± 0.22	370.72 ± 8.23 <sup>b</sup>	1026.25 ± 16.00 <sup>bc</sup>	1427.56 ± 15.29	2111.64 ± 38.40	
5	-	+	+	-	38.47 ± 0.39	402.38 ± 4.78 <sup>a</sup>	1079.92 ± 13.66 <sup>ab</sup>	1441.74 ± 24.93	2213.49 ± 25.99	
6	-	+	-	+	37.74 ± 0.42	399.91 ± 3.41 <sup>a</sup>	1093.85 ± 15.13 <sup>a</sup>	1482.42 ± 23.40	2186.09 ± 36.78	

\*Values represent the mean ± SEM of six group of 90 broiler chicks (six replicates of 15 broilers in each) per treatment; <sup>a-c</sup>Means within columns with no common superscripts are significantly different (P < 0.001), according to Duncan's multiple range tests. \*\*Age (days).

**TABLE 4: Effect of Boron (B) and vitamin D<sub>3</sub> on food consumption (fc) for broiler chicks\***

Group number	Treatment				Food consumption (g day <sup>-1</sup> )						
	B mg kg <sup>-1</sup>		Vit D <sub>3</sub> IU kg <sup>-1</sup>		1-14**	1-28	1-35	1-45	14-28	28-45	35-45
	5	25	250	2000							
1	-	-	+	-	40.28 ± 1.17 <sup>ab</sup>	58.57 ± 0.66	71.65 ± 0.95 <sup>b</sup>	89.00 ± 1.06	76.87 ± 1.14	139.41 ± 2.42 <sup>b</sup>	150.55 ± 3.42 <sup>b</sup>
2	-	-	-	+	37.74 ± 1.24 <sup>ab</sup>	60.00 ± 1.96	72.70 ± 1.85 <sup>b</sup>	90.22 ± 1.84	82.26 ± 3.36	140.00 ± 1.96 <sup>b</sup>	151.56 ± 2.21 <sup>b</sup>
3	+	-	+	-	41.03 ± 1.68 <sup>a</sup>	62.28 ± 1.50	78.20 ± 1.60 <sup>a</sup>	93.01 ± 1.45	83.70 ± 2.47	144.27 ± 1.29 <sup>ab</sup>	152.06 ± 2.06 <sup>b</sup>
4	+	-	-	+	37.06 ± 0.85 <sup>b</sup>	58.99 ± 0.85	72.88 ± 0.88 <sup>b</sup>	92.80 ± 0.85	80.94 ± 1.67	149.80 ± 1.43 <sup>a</sup>	163.85 ± 1.92 <sup>a</sup>
5	-	+	+	-	41.07 ± 0.61 <sup>a</sup>	60.53 ± 1.06	74.04 ± 1.01 <sup>b</sup>	91.06 ± 1.19	80.19 ± 2.15	142.32 ± 2.54 <sup>b</sup>	151.49 ± 2.23 <sup>b</sup>
6	-	+	-	+	38.73 ± 0.55 <sup>ab</sup>	59.25 ± 1.09	72.70 ± 1.20 <sup>b</sup>	91.16 ± 1.45	79.76 ± 1.75	143.73 ± 2.65 <sup>ab</sup>	155.78 ± 3.56 <sup>b</sup>

\* Values represent the mean ± SEM of six group of 90 broiler chicks (six replicates of 15 broilers in each) per treatment; <sup>a, b</sup>Means within columns with no common superscripts are significantly different (P < 0.05), according to Duncan's multiple range tests; \*\* Age (days).

**TABLE 5: Effect of Boron (B) and vitamin D<sub>3</sub> on food conversion ratio (FCR) for broiler chicks\***

Group number	Treatment				Food conversion ratio (kg food kg BW <sup>-1</sup> )						
	B mg kg <sup>-1</sup>		Vit D <sub>3</sub> IU kg <sup>-1</sup>		1-14**	1-28 <sup>x</sup>	1-35	1-45	14-28 <sup>x</sup>	28-45	35-45
	5	25	250	2000							
1	-	-	+	-	1.74 ± 0.04 <sup>a</sup>	1.73 ± 0.02 <sup>ab</sup>	1.90 ± 0.02 <sup>ab</sup>	1.94 ± 0.02	1.72 ± 0.02 <sup>abc</sup>	2.12 ± 0.04 <sup>b</sup>	2.02 ± 0.04
2	-	-	-	+	1.65 ± 0.03 <sup>ab</sup>	1.76 ± 0.04 <sup>a</sup>	1.90 ± 0.04 <sup>ab</sup>	1.95 ± 0.02	1.81 ± 0.06 <sup>a</sup>	2.11 ± 0.01 <sup>b</sup>	2.06 ± 0.03
3	+	-	+	-	1.59 ± 0.06 <sup>bc</sup>	1.70 ± 0.04 <sup>abc</sup>	1.99 ± 0.08 <sup>a</sup>	2.02 ± 0.05	1.77 ± 0.05 <sup>ab</sup>	2.34 ± 0.09 <sup>ab</sup>	2.21 ± 0.10
4	+	-	-	+	1.57 ± 0.04 <sup>bc</sup>	1.68 ± 0.02 <sup>bc</sup>	1.84 ± 0.01 <sup>bc</sup>	2.02 ± 0.05	1.73 ± 0.03 <sup>ab</sup>	2.37 ± 0.12 <sup>a</sup>	2.29 ± 0.11
5	-	+	+	-	1.58 ± 0.04 <sup>bc</sup>	1.63 ± 0.02 <sup>cd</sup>	1.85 ± 0.02 <sup>bc</sup>	1.89 ± 0.04	1.66 ± 0.03 <sup>bc</sup>	2.15 ± 0.08 <sup>b</sup>	2.08 ± 0.09
6	-	+	-	+	1.50 ± 0.01 <sup>c</sup>	1.57 ± 0.01 <sup>d</sup>	1.76 ± 0.01 <sup>c</sup>	1.91 ± 0.02	1.61 ± 0.01 <sup>c</sup>	2.24 ± 0.05 <sup>ab</sup>	2.22 ± 0.06

\* Values represent the mean ± SEM of six groups of 90 broiler chicks (six replicates of 15 broilers in each) per treatment; <sup>a-c</sup>Means within columns with no common superscripts are significantly different (P < 0.05) and <sup>x</sup>(P < 0.001), according to Duncan's multiple range tests; \*\*Age (days).

**TABLE 6: Effect of Boron (B) and vitamin D<sub>3</sub> on serum biochemical parameters for broiler chicks at day 45\***

Group number	Treatment				Serum concentrations				
	B mg kg <sup>-1</sup>		Vit D <sub>3</sub> IU kg <sup>-1</sup>		Ca (mg dl <sup>-1</sup> )	P (mg dl <sup>-1</sup> )	Mg (mg dl <sup>-1</sup> )	Glucose (mg dl <sup>-1</sup> )	AP IU L <sup>-1</sup>
	5	25	250	2000					
1	-	-	+	-	7.44 ± 0.63 <sup>a</sup>	3.06 ± 0.28 <sup>b</sup>	1.70 ± 0.18	234 ± 6.71	161 ± 5.08 <sup>a</sup>
2	-	-	-	+	6.23 ± 0.40 <sup>ab</sup>	3.33 ± 0.31 <sup>b</sup>	1.81 ± 0.1	234 ± 4.60	141 ± 6.51 <sup>b</sup>
3	+	-	+	-	7.18 ± 0.63 <sup>a</sup>	2.91 ± 0.51 <sup>b</sup>	1.34 ± 0.10	232 ± 8.59	135 ± 3.35 <sup>b</sup>
4	+	-	-	+	6.35 ± 0.47 <sup>ab</sup>	3.40 ± 0.11 <sup>ab</sup>	1.77 ± 0.08	236 ± 6.20	111 ± 2.67 <sup>cd</sup>
5	-	+	+	-	5.16 ± 0.27 <sup>b</sup>	3.33 ± 0.40 <sup>ab</sup>	1.61 ± 0.14	235 ± 4.32	105 ± 2.46 <sup>d</sup>
6	-	+	-	+	5.59 ± 0.33 <sup>b</sup>	3.71 ± 0.72 <sup>a</sup>	1.54 ± 0.11	246 ± 3.89	121 ± 6.95 <sup>c</sup>

\*Values represent the mean ± SEM of six groups of 12 broiler chicks each per treatment; <sup>a-b</sup>Means within columns with no common superscripts are significantly different (P < 0.05), according to Duncan's multiple range tests; <sup>a-c</sup>Means within columns with no common.

adequate D<sub>3</sub> diet containing either 60 or 120 mg kg<sup>-1</sup> of B. This may be explained by the observations in broiler chicks of Rossi et al (1990, 1993) and Wilson and Ruszler (1996, 1997, 1998) on laying hens, that high levels of B intake suppressed FC.

It is of interest that in the first few weeks of life the BW, FC and FCR were all best in the groups with high levels of both supplements and also low D<sub>3</sub> with high boron levels.

Boron seems to have a regulatory role in mineral metabolism but the mechanism of action is not understood. It is possible that the B and Ca interaction is influenced by hormone function with alteration of cell membrane or trans-membrane signalling (McDowell 1992). Boron deprivation elevates plasma AP and modulates hepatic glycolysis particularly when D<sub>3</sub> intake is inadequate (Nielsen and Schuler, 1992, Hunt and Nielsen, 1981 and 1987). Our results support the effect of B on Ca as indicated above, although significant differences in P values were not supportive. The decrease in serum Ca values indicates that reduced mobilisation of Ca from bone and increased AP activity are related to bone catabolism especially with D<sub>3</sub> deficiency (Hegsted et al 1991, McDowell 1992).

In general, our results regarding the influence of B on Ca and AP especially in low levels of D<sub>3</sub> support the findings of others mentioned above, including those of Hunt et al (1983), Hunt (1989), Nielsen (1992) and Hunt and Nielsen (1981). They also emphasise the value of further studies on the effects of B on the growth of broilers and bone metabolism.

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