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INFLUENCE OF VITAMIN E DIETARY SUPPLEMENTATION ON SOME BONE CHARACTERISTICS IN BROILER CHICKS

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SUMMARY

One hundred and sixty, commercial, one day old, Cobb chicks were randomly allocated into four groups each of forty. The first group was fed on a control diet. The second group was fed on the control diet in which 250 mg vitamin E/kg diet was added. The third group was fed on the control diet in which 500 mg vitamin E/kg diet was added while the fourth group was fed on a control diet in which 750 mg vitamin E/kg diet was added. Several parameters were assessed including body weight development, weight gain, feed consumption and conversion, serum parathyroid hormone and calcitonin, bone Ca, P, S, Mg as well as Mn. Tibia length and weight as well as scanning electron microscope examination of the tibia were recorded. Results indicted that vitamin E supplementation significantly improved body weights starting from the first week of the study throughout the experimental period. All treated groups had better weight gain and feed conversion. Vitamin E supplementation at a level of 250 mg/kg diet significantly ($P< 0.05$) increased bone P and Mg. Moreover, vitamin E supplementation at a level of 500 mg/kg significantly ($P< 0.05$) increased bone Ca, P, S as well as Mg. In addition, vitamin E supplementation at a level of 750 mg/kg significantly ($P< 0.05$) increased bone Ca, P, S, Mg as well as Mn. None of the used levels of vitamin E had any effect on tibia length 7, 28 and 60 days from the beginning of the study. The three used levels of vitamin E significantly ($P<0.05$) increased the tibia weight compared to the control group at the age of 60 days. At the age of 28 days, vitamin E supplementation at levels of 500 and 750 significantly ($P<0.05$) increased serum calcitonin, while no significant effect was found regarding parathyroid hormone. Moreover, at the age of 56 days, vitamin E and only at a level of 750 mg/kg diet significantly ($P<0.05$) increased serum calcitonin and also no significant

effect was found regarding parathyroid hormone. The scanning electron micrograph examination of tibia revealed a positive impact of supplemental vitamin E on bone characteristics.

Key Words: Vitamin E, broiler, bone characteristics, calcitonin, parathyroid hormone.

INTRODUCTION

Vitamin E is recognized as an essential nutrient for all species of animals including human and birds. It is well established that it has a number of functions including its biological antioxidant effect, its role in membrane structure and prostaglandin synthesis (Meydani et al., 1991; Sakamoto et al., 1991, Beharka et al., 1997; Qureshi and Gore 1997; Watkins and Chen 1997 and Wu et al., 1998), blood clotting (McDowell 1989), disease resistance and immune status (Mohamed 1988; Mohamed et al., 1990; Franchini et al., 1990; Franchini et al., 1995, Mohamed 1998 as well as Osman 1999), its role in electron transport and biosynthesis of DNA within cells, its protection against some toxic elements, tissue protection and several other functions (McDowell 1989).

Bone growth and modeling are regulated by complex interactions between an individual's genetic potential, environmental influences and nutrition. These interactions produce a bone architecture that balances functionally appropriate morphology with the skeleton's role in calcium and phosphorus homeostasis (Watkins, 1998). Recent studies indicated that vitamin E benefits bone growth and cartilage activity. Chicks supplemented with vitamin E demonstrated higher bone formation rates (Xu et al., 1995). It increased bone volume in rat model of iron-induced free radical impairment of bone formation (Ebina et al., 1991). Vitamin E may diminish oxygen-derived free radical stimulation of osteoclastic bone resorption (Garrett et al., 1990) as evidenced by reduced vascular invasion of growth cartilage in chicks (Xu et al., 1995), and by decreased osteoclast number and percent surface in the rat (Ebina et al., 1991). Vitamin E restored collagen synthesis in injured chondrocytes (Watkins et al., 1996). The decrease in collagen synthesis which appears to be related to membrane damage and impaired cell function, for which vitamin E is protective (Watkins (1998). The current study was conducted to shed light and confirm the possible role and impact of vitamin E on some bone characteristics in broiler chickens.

MATERIAL AND METHODS

One hundred and sixty, commercial, one day old, Cobb chicks were randomly allocated into four groups each of forty. The first group was fed on a control diet. The second group was fed on the control diet plus 250 mg vitamin E/kg diet. The third group was fed on the control diet plus 500 mg vitamin E/kg diet while the fourth group was

fed on a control diet plus 750 mg vitamin E/kg diet. The chicks were floor reared in an electrically heated room provided with clean feeders and waterers and kept under suitable hygienic and managemental conditions. The birds were fed ad-libitum with constant access to fresh water. The birds were fed on unmedicated starter(day one-3 weeks of age) and finisher (3-8 weeks of age) diets (Table 1) to serve as basal diets to which vitamin E was added.

Birds were weighed individually every week. Weight gain and feed/gain were calculated. Feed consumption was recorded weekly. Bone minerals (Ca, P, S, Mg and Mn) were determined according to the standard methods described by AOAC (1990).

The parathyroid hormone was determined by the Diagnostic System Laboratories (DSL) 7700 using C-PTH radioimmunoassay kit according to the method described by Talow and Berson (1971). Serum calcitonin was assayed by DSL-7700 using calcitonin coated tube immunoradiometric assay kit as described by Miles et al., (1974). The hormonal assays were performed in the laboratories of the Atomic Energy Authority, Cairo, Egypt. The parathyroid hormone and calcitonin were determined at 28 and 56 days of age.

The right tibia from each bird was excised and defleshed without boiling. The tibias were individually sealed in a plastic bag to minimize moisture loss, and stored at -20 C until analysis. Storage of wet bone at -20 C has been reported to have no effect on various bone parameters (Zhang and Coon 1997). The tibia weight was determined with an accuracy of 0.001 g and the tibia length was measured with a caliper with an accuracy of 0.001 cm. The tibia length and weight were determined at 7, 28 and 60 days of age.

For scanning electron microscope examination of tibia, after fixation, the tissue was dehydrated in ascending grades of alcohol, dried by the critical point method with liquid carbon dioxide in a critical point drier, mounted on stubs and coated with gold (Bancroft and Stevens, 1982). The processed tissues were examined using a JEOL, J.S. M., 25. S 11. Scanning microscope at Faculty of Agriculture, Alexandria University. Sections were made from tibial cortical surface, tibial medullary surface as well as the intermediate area between the cortical and medullary surfaces.

The obtained data were analyzed by One Way Analysis of Variance with Newman-Keuls post test using Graph Pad Prism Software (1999).

RESULTS AND DISCUSSION

The data of the body weight development are shown in table (2). Results indicated that vitamin E supplementation significantly improved body weights starting from the first week of the study throughout the experimental period. Data of total

body gain, feed consumption and conversion (Table 3) showed that all treated groups had better weight gain and feed conversion. Less feed consumption was recorded in groups treated with 500 and 750 mg vitamin E/kg diet. Similar results were reported by Batrov and Frigg (1992); Kennedy et al., (1992) Ajuyah et al., (1993) as well as Sell et al., (1995). The improvement in body weight development in birds supplemented with vitamin E could be attributed to the improved feed conversion which further could be attributed to the biological function (s) of vitamin E such as its role in enzymatic oxidation reduction, nucleic acid metabolism and in promoting the activity of easily oxidized substances such as carotenoids and vitamin A (Osman, 1999). Moreover, such improvement may be due to the role of vitamin E as an immuno-enhancer (Mohamed 1988; Franchini et al., 1990; Franchini et al., 1995 as well as Osman 1999) which in turn raises the bird's resistance.

Palm vitamin E and alpha-tocopherol maintained bone mineral density in ovariectomised rats (Norazlina et al., 2000). The effect of different levels of vitamin E supplementation on different bone minerals is shown in table (4). Results indicated that vitamin E supplementation at a level of 250 mg/kg diet significantly ($P< 0.05$) increased bone Mg. Moreover vitamin E supplementation at a level of 500 mg/kg significantly ($P< 0.05$) increased bone Ca, P, S as well as Mg. In addition, vitamin E supplementation at a level of 750 mg/kg significantly ($P< 0.05$) increased bone Ca, P, S, Mg as well as Mn. The increased bone mineral levels due to vitamin E supplementation could be explained by the fact that vitamin E increases the mineral apposition rate in chicks fed supplemental vitamin E (Xu et al., 1995; Watkins and Chen 1997).

The effect of dietary supplementation with different levels of vitamin E on tibia bone length and weight is shown in table (5). The obtained data indicated that non of the used levels of vitamin E had any effect on tibia length 7, 28 and 60 days from the beginning of the study. Similar results were reported by Xu et al., (1995) and Maiorano et al., (1999). In regard to tibia weight, non of the three used levels of vitamin E had any impact on the tibia weight 7 and 28 days from the beginning of the study but this is not the case at 60 days from the beginning of the study as the three used levels of vitamin E significantly ($P< 0.05$) increased the tibia weight compared to the control group.

Bone formation and bone resorption are regulated by systemic hormones and factors produced locally primarily by osteoblasts (Watrous and Andrews 1989; Baylink et al., 1993; Raisz, 1993). Systemic hormones involved in stimulating bone formation

include insulin, growth hormone (Nilsson et al., 1994), as well as estrogen (Chow et al., 1992); while those involved in stimulating hone resorption include. 1 , 25-(OH) 2 vitamin D3 (Raisz 1990), PTH (Kream et al., 1990), and thyroid hormone (Klaushofer et al., 1989). In addition, calcitonin (Lin et al., 1991) and glucocorticoids (Lukert and Raisz 1990) inhibit bone resorption.

Tocopherol deficiency adversely affected the ultrastructure of calcitonin secreting C-cells of the thyroid gland of rats (Blähser and Schnorr 1972). Moreover, the serum blood levels of the parathyroid hormone (PTH) under E-avitaminosis was significantly lowered (Sergeev et al., 1990). The data presented in table (5) shows the effect of dietary supplementation with vitamin E on serum calcitonin and parathyroid hormone. At the age of 28 days, vitamin E supplementation at levels of 500 and 750 and (not 250 mg/kg diet) significantly ($P<0.05$) increased serum calcitonin, while no significant effect was found regarding parathyroid hormone. Moreover, at the age of 56 days, vitamin E and only at a level of 750 mg/kg diet significantly ($P<0.05$) increased serum calcitonin and also no significant effect was found regarding parathyroid hormone.

Bone growth includes the activities of bone matrix formation, matrix mineralization, and bone resorption. Bone matrix is produced and mineralized through the activity of osteoblasts while bone matrix resorption is accomplished by osteoclasts (Baron, 1993). The combined and cooperative activities of osteoblasts and osteoclasts result in a bone architecture that provides mechanical support and maintains normal serum concentrations of calcium and phosphorus (Watkins 1998). Recent reports suggest that vitamin E is a crucial factor in bone growth and cartilage activity. Chicks given supplemental vitamin E demonstrated higher bone formation rates (Xu et al., 1995), it increased bone volume in a rat model of iron-induced free radical impairment of bone formation (Ebina et al., 1991) and diminished oxygen-derived free radical stimulation of osteoclastic bone resorption (Garrett et al., 1990) as evidenced by reduced vascular invasion of growth cartilage in chicks (Xu et al., 1995), and by decreased osteoclast number and percent surface in the aforementioned rat model. Ima-Nirwana et al., (1999) studied the effect of palm oil vitamin E on bone turnover in thyrotoxic rats and reported a reduced bone resorption to a greater extent than bone formation suggesting a net reduction in bone loss. Moreover, vitamin E deficiency in vivo resulted in the inhibition of vitamin D metabolism in the liver and kidney concomitant with the formation of active metabolites and decreases the concentration of hormone-receptor complexes in target tissues (Sergeev et al., 1990).

Research indicated that the mineralized area of growth plate cartilage has a limited enzymatic ca-

pacity for handling oxidized lipid species because superoxide dismutase and catalase activities are low in this region (Matsumoto et al., 1991). The enrichment of epiphyseal chondrocytes with 18:2 (w6) resulted in cellular injury (elevated lactate dehydrogenase (LDH) activity) and depressed collagen synthesis when compared to cells supplemented with oleic acid or no fatty acids and vitamin E restored collagen synthesis in these cells (Watkins et al., 1996).

In the current study, the scanning electron micrographs (SEM) of the tibia bone are shown in figures (1-12). The bone was cross sectioned in the middle and sections were made from the tibial cortical surface (C), tibial medullary surface (M) and the intermediate area between cortical and medullary surfaces (I). The SEM of the control tibia (C, I and M) are shown in figures (1-3). Figures (4-6) represent the group received 250 mg/kg vitamin E. Moderate mineralization of bony tissue (C), proliferation of osteoblasts (I), increased number of bone trabeculae (M) were observed. Figures (7-9) represent the group received 500 mg/kg vitamin E. A tendency for an increase in mineral apposition rate (C) was observed, a marked proliferation of osteoblasts (I) with an increased volume of boney trabeculae (M) were observed. Figures (10-12) represent the group received 750 mg/kg vitamin E. An increased bone mineralization was observed (C), increased osteoblasts proliferation (I) with an increased bony mineralization and a tendency for decreased trabecular separation (M) were observed.

The exact mechanism by which vitamin E improves bone characteristics is not completely understood. Several suggestions are available including its inhibition of PGE_2 synthesis (Meydani et al., 1991; Sakamoto et al., 1991; Beharka et al., 1997; Qureshi and Gore 1997 and Wu et al., 1998). The elevated production of PGE_2 caused reduction in collagen synthesis in primary cultures of epiphyseal chondrocytes and stimulated an increase in bone resorptive activity to produce a reduction in bone volume and trabecular number and an increase in trabecular separation (Watkins and Chen 1997). Another suggestion is it's effect on calcitonin. Increasing the level of calcitonin inhibits bone resorption and so leads to a net reduction in bone loss. Also by increasing the mineral apposition rate in the bone. Moreover, the fact that free radical production increases the osteoclastic activity, lipid peroxidation products may inhibit osteoblastic activity, and vitamin E as a biological antioxidant may enhance bone mass by reducing free radical concentrations that stimulate osteoclasts or depress osteoblastic bone formation (Watkins and Chen 1997).

Conclusions:

Vitamin E dietary supplementation increased body weight development, weight gain, improved

feed/gain and decreased feed consumption. It increased bone mineralization, serum calcitonin, tibia weight and several characteristics of the tibia bone as evidenced by the scanning electron microscope examination.

Implications:

The data obtained in this study suggest further studies to assess the possible role of supplemental vitamin E in laying hens. Also, it's possible role in reducing the intensity of Osteoporosis in human subjects. It is a condition of decreased bone mass due to impaired coupling between bone formation and resorption. Moreover, the condition of Rheumatoid arthritis, which is caused by excessive lipid metabolism and oxidative stress, may be alleviated by supplemental vitamin E.

Table (1): Composition and Calculated Analysis of Experimental Diets:

Ingredient	Inclusion rate %	
	Starter	Finisher
Ground yellow corn	58.08	63.96
Soybean meal (44 % CP)	32.73	28.67
Fish meal (72.3 % CP)	2.50	-
Meat Meal (52 % CP)	1.14	2.31
Dicalcium phosphate	1.21	1.15
Limestone	1.35	0.83
NaCl	0.35	0.35
Vitamin and Mineral Premix *	0.20	0.20
Synthetic methionine	0.13	0.18
Vegetable oil	2.31	2.35
Calculated analysis:		
Crude Protein %	22.5	20.0
True Metabolizable Energy Kcal/kg	3050	3100
Calorie/protein ratio	135.55	155
Crude fat %	5.0	5.0
Crude fiber %	2.91	2.92
Calcium %	1.06	0.85
Available Phosphorus %	0.45	0.42
Sodium %	0.22	0.22
Lysine %	1.34	1.13
Methionine %	0.53	0.51
Methionine + Cystine	0.9	0.85
Arginine %	1.61	1.43
Linoleic acid %	2.61	2.71

* Supplied per kg of diet: Vitamin A: 6600 IU; Vitamin D_3: 2200 IU; B_2: 4.4 mg; Pantothenic acid: 13.2 mg; Niacin, 39.6 mg; Choline chloride: 500 mg; B_{12}: 0.022 mg; Mn: 0.55 mg; Fe: 50 mg; Cu: 4 mg; Zn: 40 mg.

Table (2): Effect of Dietary Supplementation with Vitamin E on Body Weight Development in Broilers.

Group / Age	Control	Vitamin E 250 mg/kg diet	Vitamin E 500 mg/kg diet	Vitamin E 750 mg/kg diet
Initial Weight (Zero day)	43.09 ± 0.65	45.64 ±0.71	46.46 ±0.75	47.40 ±0.66
First Week	149.0^{a} ±3.95	160.5^{b*} ±3.32	173.2^{b**} ±3.42	173.8^{b**} ±3.61
Second Week	320.1^{a} ±11.74	347.8 ^{b*} ±7.88	352.9^{b*} ±7.29	376.6 ^{b**} ±6.30
Third Week	593.3^{a} ±17.2	639.4 ^{b*} ±9.2	632.4^{b*} ±7.86	675.6^{b**} ±7.99
Fourth Week	970.6^{a} ±36.65	1084.0^{b*} ±21.68	1088.0^{b*} ±25.58	1093.0 ^{b**} ±17.65
Fifth Week	1353.0^{a} ±36.54	1471.0 ^{b*} ±30.97	1479.0 ^{b*} ±31.65	1523.0^{b**} ±29.44
Sixth Week	1667.0^{a} ±61.08	1866.0 ^{b*} ±54.75	1909.0^{b*} ±40.14	1950.0 ^{b**} ±65.29
Seventh Week	1798.0^{a} ±77.22	2026.0 ^{b*} ±63.63	2092.0^{b*} ±71.85	2120.0 ^{b*} ±76.27
Eighth Week	1942.0^{a} ± 83.82	2208.0 ^{b*} ±80.96	2214.0^{b*} ±60.93	2282.0 ^{b*} ±51.47

Values are means ± SE of the mean.

Values in the same row with different superscript vary significantly at: * $P \leq 0.05$; ** $P < 0.01$.

Table (3): Effect of Dietary Supplementation with Vitamin E on Overall Performance of Broilers

	Control	Vitamin E 250 mg/kg diet	Vitamin E 500 mg/kg diet	Vitamin E 750 mg/kg diet
Initial Wt. (g.)	43.09	45.64	46.46	47.40
Final Wt. (g.)	1942.0	2208.0	2214.0	2282.0
Total Gain (g.)	1898.91	2162.36.	2167.54	2234.6
Total Feed Consumed (g.)	4557.38	4584.2	4335.08	4245.74
Feed:Gain Ratio	2.4	2.12	2.00	1.90

Table (4): Effect of Dietary Supplementation with Vitamin E on different bone minerals of Broiler chicks (mg/g).

	Ca	P	S	Mg	Mn
Control	90.7[a] ±0.26	63.78[a] ±0.87	1.31[a] ±0.3	5.32[a] ±0.14	0.76[a] ±0.01
VitaminE 250 mg/kg	93.14[a] ±0.32	58.5[b] ±0.63	1.93[a] ±0.04	7.0[b] ±0.41	0.82[a] ±0.01
Vitamin E 500 mg/kg	102.1[b] ±3.78	68.96[c] ±0.48	3.24[b] ±0.11	8.14c ±0.26	0.88b[a] ±0.01
Vitamin E 750 mg/kg	109.0[c] ± 0.20	69.95[c] ±0.24	4.37[c] ±0.47	12.11[d] ±0.32	0.96[c] ±0.04

Values are means ± SE of the mean.
Values in the same column with different superscript vary significantly at P≤0.05.

Table (5): Effect of Dietary Supplementation with Vitamin E on Tibia bone Length and Weight of Broilers.

	Tibia length (cm)			Tibia weight (g.)		
	7 days	28 day	60 day	7 day	28 day	60 day
Control	4.90 ±0.1	8.23 ±0.08	11.6 ±0.40	1.87 ±0.14	9.08 ±0.40	18.90[a] ±0.27
VitaminE 250 mg/kg	4.76 ±0.06	9.3 ±0.69	11.95 ±0.37	1.85 ±0.16	8.62 ±0.32	21.86[b] ±0.22
Vitamin E 500 mg/kg	4.9 ±0.11	8.25 ±0.12	11.93 ±0.22	1.70 ±0.18	9.83 ±0.12	23.95[c] ±1.35
Vitamin E 750 mg/kg	5.03 ±0.08	8.26 ±0.14	12.18 ±0.34	2.15 ±0.05	9.09 ±0.75	25.51[d] ±1.3

Values are means ± SE of the mean.
Values in the same column with different superscript vary significantly at P≤0.05.

Table (5): Effect of Dietary Supplementation with Vitamin E on serum calcitonin and parathyroid hormone of Broilers.

	serum calcitonin pg/ml		parathyroid hormone ng/ml	
	28 days	56 day	28 days	56 day
Control	126.3 ±3.28	140.0 ±5.508	1.757 ±0.2794	1.077 ±0.091
VitaminE 250 mg/kg	136.7 ±4.63	208.3 ±32.65	1.423 ±0.0809	1.180 ±0.26
Vitamin E 500 mg/kg	45.7* ±1.63	202.7 ±10.73	1.343 ±0.2720	0.5967 ±0.04
Vitamin E 750 mg/kg	176.7* ±4.66	254.0* ±7.810	1.037 ±0.1224	0.5233 ±0.08

Values are means ± SE of the mean.

* significantly differ at $P \leq 0.05$ compared to control.

Scanning Electron Microscope Examination:

Figure (1): Scanning electron micrograph of the cortical surface of the tibia of the control group. (X100).

Figure (2): Scanning electron micrograph of the intermediate zone between cortical and medullary surfaces of the tibia of the control group. (X300).

Figure (3): Scanning electron micrograph of the medullary surface of the tibia of the control group. (X100).

Figure (4): Scanning electron micrograph showing moderate mineralization of bony tissue (X100).

Figure (5): Scanning electron micrograph showing proliferation of osteoblasts (X300).

Figure (6): Scanning electron micrograph showing increased number of bone trabeculae. (X300).

Figure (7): Scanning electron micrograph showing tendency for an increase in mineral apposition rate. (X100).

Figure (8): Scanning electron micrograph showing marked proliferation of osteoblasts (X300).

Figure (9): Scanning electron micrograph showing an increased volume of boney trabeculae (X100).

Figure (10): Scanning electron micrograph showing an increased bone mineralization. (X100).

Figure (11): Scanning electron micrograph showing increased osteoblasts proliferation. (X300)

Figure (12): Scanning electron micrograph showing increased bony mineralization and a tendency for decreased trabecular separation (X100).

Figures (1-12): Scanning Electron Micrographs (SEM) of Tibia Bone as influenced by supplemental vitamin E

Figure 1

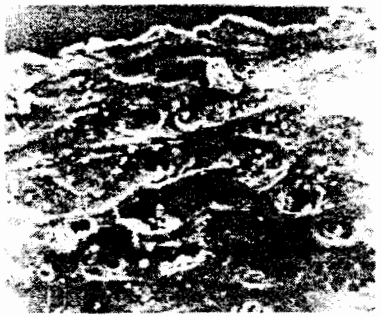

Figure 4

Figure 7

Figure 10

Figure 2

Figure 5

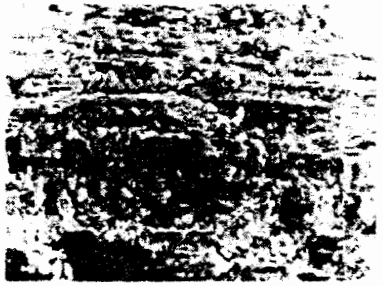

Figure 8

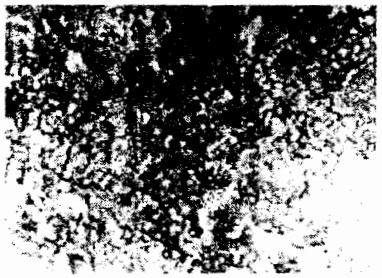

Figure 11

Figure 3

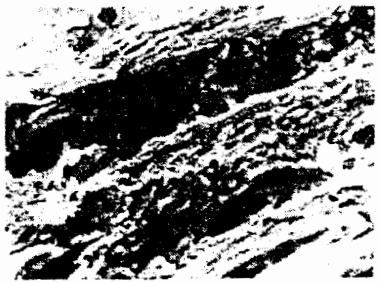

Figure 6

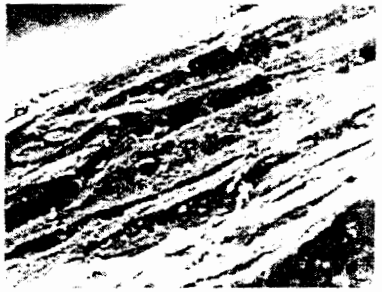

Figure 9

Figure 12

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