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ROLE OF DIETARY ZINC ON IMMUNE RESPONSE OF BROILER CHICKENS

BY

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INTRODUCTION

The importance of dietary factors in modulating immune responeses and altering susceptibility to infections and other illnesses has only recently been studied in a systemic manner. Many nutrients regulate lymphoid cellular activity, and both deficiency and excess can produce adverse functional consequences.

Specific nutrient imbalance varies in the ability to influence the immune response and the mechanisms underlying the immunological abnormalities. Among others, zinc deficiency is associated with marked immunodepression (Fernandes et al. 1979). In fact, the thymus appears to be the most atrophied organ in zinc deficient animals (Golden et al. 1978). Because the thymus is important immunological competence, this effect of zinc has received considerable research attention in recent years (Cohen and Duke 1984).

In birds, a similar result was obtained by Weight et al. (1980), who observed a severe monocytosis in zinc-deficient chickens, however, the affected birds showe prominent growth retardation, pedal dermatities and

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caseous encrustations of the external mucous surface. Burns (1983) observed no antibody response as judged by immunoelectrophoresis and immunefluorecence in zinc-deficient chick, whereas, birds on zinc-sufficient diet had higher serum antibody and anti-bovine serum albumin cells in Harderian gland, spleen and thymus. The author found that localization of immunoglobulins did not differ between control and deficient birds except in the bursa of Fabricius. Shoyinka and Daudu (1986) revealed a higher HI values with the usage of 200 mg sinc/kg diet in Harco- Cockerels as well as in group of chicks raised on diet supplemented with 400 mg zinc/kg diet. However, Beisal (1980) had reported that very low or very high concentration of zinc in diet may impair the host's resistance.

Researches have shown that chickens require zinc as a nutrient for gorwth as well as normal feathering and skin condition (O'Dell and Savage, 1957; Roberson and Schaible, 1975). Robarson and Schaible (1958) indicated that zinc supplementation at 100 ppm improved the average 4-weeks weight and efficiency of food utilization in male chicks. However, the same workers in (1959) observed that high zinc level up to 1000 ppm as oxide, sulfate or carbonate did not significantly affect the rate of growth, feed efficiency and livability.

It seems that zinc requirement is influenced greatly by several dietary factors among them the calcium-zinc-phytate interaction. O'Dell and Savage (1960) demonstrated that phytic acid as a component of natural corn-soya diets had impaired zinc bioavailability in chicks. O'Dell et al. (1964) also reported that excess of dietary calcium might aggravate zinc deficiency in phytate containing diets marginally adequate in zinc. Bafundo et al. (1984) found that both excess dietary Ca (0.91 or 1.84%) and phytate addition to diet markedly reduced plasma zinc concentration, zinc deposition in tissues and performance of birds fed corn-Soya bean meal diets (33 zn/kg) unsupplemented with zinc. Addition of zinc at rate of 52 mg/kg diet, however compensate the impaired effect of excess Ca and phytate.

Dietary zinc deficiency occurs fairly frequently in poultry whose diet consists largely of grains and cereals. Such a diet apart from being low in zinc, contains also high amounts of phytate and fiber that adversly affect the bioavailability of zinc.

The aim of the present study was to find out the possible role of dietary zinc on performance of growing in relation to Newcastle disease vaccine and challenge. Moreover the effect of dietary zinc on the relative weights of certain immune-lymphoid organs such as spleen, cecal tonsile, Harderian gland and bursa was studied.

MATERIALS AND METHODS

I- Materials

1. Experimental birds:

Two hundred and twenty one-day Hubbard broiler chicks of approximately uniform size were allocated into five experimental groups. Each group was housed in seperate ellectrically heated rearing unite and subjected to different treatment (Table 2).

2. Experimental diets:

The composition and analysis of main experimental diets (A & B) are presented in Table (1). Both diets A & B were based on corn-soya bean meal, animal protein-free diet. Diet A was formulated with high calcium level (approximately 1.5 times the normal requirement) to impair zinc bioavailability in chicks (group 1). Diet B was formulated to contain normal Ca requirement. Diet B group was divided into 4 experimental groups according to zinc levels as shown in Table (2). Feed and water were acessed ad libitum in plastic feeders and wateres.

Table (1): Compsition and analysis of the experimental diets (A & B).

Type of diet	A	B
Amount / Ingredients	Kg	Kg
Ground yellow corn	55.2	57.0
Ground wheat	5.0	5.0
Soybean meal (44 %)	35.0	35.0
Bone meal	2.6	1.8
Salt	0.2	0.2
Limestone	1.9	0.9
Vitamin premix	0.1	0.1

*** Calculated analysis:**

	A	B
° Crude protein %	20.78	20.93
° Metabolizable energy Kcal/kg	2799.6	2856.9
° Calcium %	1.6	0.98
° Zinc mg/kg	15.1	15.3
° Analysed zinc mg/kg	15.0	17.5

Table (2): Treatment used for each experimental group

group number	Tratment	zn content (mg/kf diet)	Vaccination programme against ND	challenge against NDV
1	A	15	+ **	+
2	B	17.5	+	+
3	B+150mg zno/kg	137.95*	+	+
4	B+ 50mg zno/kg	57.65*	+	+
5	B+ 50mg zno/kg	57.65*	-	+

* Based on the molecular weight of zinc oxide (ZnO) and percentage of zinc in it (80.3 %9 + the zinc content of basal diet.

**+ Vaccinated - unvaccinated

3. Newcastle disease challenge virus

A velogenic ND challenge virus strain identified by Sheble and Reda (1976) was used after titeration in challenge test.

II. Methods:

- Determination of virus infectivity: Titration of living virus before use was carried out according to Anon (1971), while the EID was calculated according to Reed and Muench (1938).

- Haemagglutination inhibition test: (HI test) was done following the beta procedure in microtitre plates after Takatsy (1956).

- Challenge test:

Birds of group (1-4) were challenged against velogenic Newcastle disease virus strain by Intramuscular inoculation with 0.2 ml/bird containing 10^6 EID_{50} at age of 8-weeks.

- Vaccination programe against ND:

All chicks in group (1-4) were vaccinated with Hitchner B_1 vaccine (Smithkline, PL 25301/4A) containing $10^{9.4}$ EID_{50}/ml by ocular route. At age of 21-days, the same groups were revaccinated with lasota vaccine (Delvax Labs, lot No. 38611) containing $10^{9.4}$ EID_{50}/ml via drinking water.

- Measurements:

Average body weight and feed intake were determined at weekly interval, while feed efficiency was calculated.six chicks were selected randomly from each group at weekly intervals and bled for serological analysis until the age of 8-weeks when the challenge with virlesr ND virus was carried out. The relative weights of the different lymphoid organs were determined weekly as the birds were slaughtered starting from the 4th week of age till the 8th week. Calcium and zinc were determined in diet using atomic absorption septrophotometric technique.

RESULTS AND DISCUSSION

In addition to registering the symptoms of deficiency exhibited by group 1, statistical analysis of body weight development and feed efficiency are given in Tables (3) and (4) as well as result of haemagglutination inhibition (HI) antibody titres and challenge test aganist ND virus in Table (5). Table (6), shows the relative weight of the different lymphoid organs of the chicks in different experimental groups.

Groups 2,3,4, and 5 had in general thrifty appearence. On the other hand, group 1 showed symptoms of zinc deficiency manifested by abnormal feathering, hock enlargement and higher mortalities.

The obtained results (Table 3) indicated that the addition of zinc in both levels 137.95 and 57.65 mg/kg of corn-soyabean meal diet had significantly ($P < 0.05$) increased the 8-weeks body weight, being 1181.1 and 1183.5 gm for group 3 and 4, respectively. Moreover, the chickens fed on zinc adequate diet (groups-43).

Table (3): Average body weight development (g) of chickens in different experimental groups

Age in weeks \ Group No.	1	2	3	4
0	48.0	44.9	47.9	46.4
1	87.8 ± 15.1^{ab}	93.1 ± 13.8^{c}	97.2 ± 13.3^{a}	100.3 ± 12.7^{cb}
2	110.4 ± 18.7^{ac}	128.2 ± 22.7^{b}	140.7 ± 23.9^{ab}	135.6 ± 26.8^{c}
3	-	-	-	-
4	455.8 ± 142.9^{a}	501.8 ± 136.3	499.8 ± 91.9	523.3 ± 85.9^{a}
5	590.2 ± 65.0^{abc}	686.2 ± 97.6^{c}	720.3 ± 121.9^{b}	735.4 ± 118.8^{a}
6	718.4 ± 93.7^{abc}	845.1 ± 96.6^{bc}	830.3 ± 95.7^{cd}	898.1 ± 76.0^{ade}
7	875.6 ± 173.3^{abc}	987.0 ± 139.3^{c}	989.2 ± 117.0^{b}	1002.2 ± 146.2^{a}
8	985.3 ± 62.4^{abc}	1083.9 ± 142.8^{cde}	1181.1 ± 173.9^{be}	1183.5 ± 127.1^{ad}

$\pm$ SD

* Values in the some raw with the same superscriptes are significantly different at $P<0.05$ using analysis of variance according to Snedecor (1956)

\- Unrecorded.

Table (4): Feed utilization efficiency in different groups of chicks fed on various levels of zinc.

Group No.	Total body weight gain (g)	Total feed intake (g)	Feed efficiency
1	937.3	3968.1	0.24
2	1039.0	3408.7	0.31
3	1132.2	3276.6	0.35
4	1137.1	3181.5	0.36

* Feed efficiency gain/feed (g).

achieved the best feed efficiency (0.36,0.35) during the 8-weeks experimental priod.

The improvement effect of supplemental zinc when added to low-zinc-diet on growth and feed efficiency was also reported by many workers (Roberson and Schainble 1958 and Young et al. 1958). The group of chicks (1) which received low available zinc diet, had achieved the significant ($P < 0.05$) lowest final body weight (985.3 g), the highest feed consumption (3968.1 g) and the poorer feed efficiency (0.24) compared with the other treated groups. Meanwhile, this result was associated with unthrifty condition. The previous results emphasized the suggestion of Young et al. (1958) and Bafundo et al. (1984) who revealed that with higher calcium and low zinc levels in poultry diet, zinc availability had been impaired and zinc deficiency symptoms is likely to occure.

Results in Table (5) had demonstrated that the significantly ($P < 0.05$) higher HI titres against ND virus before challenge at 8 weeks of age 5.83 recorded by group (3), fed on corn-soyabean meal diet supplemented with higher levels of zinc. The group of chicks (4), received adequate zinc diet had higher HI titres (4.5) than group (2) and (1) which were fed low available and deficient zinc diets.

Table (5): Results of haemagglutinating inhibiting (HI) antibody titres and challenge test against velogenic Newcastle disease (ND) virus

Age in days	Group No.	No. of titrated serum samples	Hianti body titres against ND virus in TRN							Geometric mean of HI titres	Protection %
			0	2	3	4	5	6	7		
1	B.G*	10	-	2	5	3	-	-	-	3.10	nd
7	(1)	6	-	3	2	1	-	-	-	2.67	38.88
14		6	2	3	-	1	-	-	-	1.67	
21		6	-	4	2	-	-	-	-	2.33	
28		6	-	3	2	1	-	-	-	2.67	
35		6	-	1	3	2	-	-	-	3.17	
42		6	1	-	2	1	2	-	-	3.33	
49		6	-	-	2	2	2	-	-	4.00	
56		6	-	1	1	1	1	2	-	4.33 b	
7	(2)	6	-	3	2	1	-	-	-	2.67	59.09
14		6	3	3	-	-	-	-	-	1.00	
21		6	1	4	1	-	-	-	-	1.83	
28		6	1	3	2	-	-	-	-	2.00	
35		6	1	2	2	1	-	-	-	2.33	
42		6	-	2	2	2	-	-	-	3.00	
49		6	-	-	1	2	2	1	-	4.50	
56		6	-	-	2	2	1	1	-	4.2 b	
7	(3)	6	-	3	2	1	-	-	-	2.67	78.57
14		6	2	3	1	-	-	-	-	1.50	
21		6	-	3	2	1	-	-	-	2.67	
28		6	-	1	3	2	-	-	-	3.17	
35		6	-	-	1	2	3	-	-	4.33	
42		6	-	-	-	2	4	-	-	4.67	
49		6	-	-	-	1	1	3	1	5.66	
56		6	-	-	-	-	1	5	-	5.83 a	
7	(4)	6	-	3	2	1	-	-	-	2.67	66.66
14		6	3	1	2	-	-	-	-	1.33	
21		6	1	3	2	-	-	-	-	2.00	
28		6	-	2	3	1	-	-	-	2.67	
35		6	-	-	4	2	-	-	-	3.33	
42		6	-	-	1	3	2	-	-	3.83	
49		6	-	-	1	2	3	-	-	4.33	
56		6	-	-	-	3	3	-	-	4.50 b	
7	(5)	6	-	3	2	1	-	-	-	2.67	0.00
14		6	4	2	-	-	-	-	-	0.67	
21		6	5	1	-	-	-	-	-	0.33	
28		6	-	-	-	-	-	-	-	0.00	
35		6	-	-	-	-	-	-	-	0.00	
42		6	-	-	-	-	-	-	-	0.00	
49		6	-	-	-	-	-	-	-	0.00	
56		6	-	-	-	-	-	-	-	0.00	

* B.G = Before grouping.

** nd = not done.

Regarding the protection percentage against the challenge with velogenic ND virus (Table 5), the results pointed out that group (3) received higher levels of supplemental zinc (137.95 mg/kg) had also recorded the best protection rate (78.57%), Whilt chicks ofgroup 4 which received 57.65 zinc kg diet and those of group that received 17.3 mg zinc / kg recorded 66.66 and 59.1% respectively. On the other hand, the group of chicks which consumed unavailable zinc diet (low zinc & high calcium) recorded the lowest protection values (38.88%).

The lowered protection recorded in both groups receiving low-available or deficient zinc diet can be supported by results obtained by Wight et al. (1980), who reported previous ultrastructural and histochemical changes in the stratified aquamous epithelia of upper alimentary tract which might lower the integrity of those tissues and allow the transit of noxious substances.

Shoginka and Dauda (1984) reported that zinc is an important component in metaloenzymes formation like thymidine kinase and DNA polymerase, both enzymes are important in protein synthesis. Zinc deficiency was known to cause decrease in immunoglobulin production differentially and this is probably due to the adverse effect of zinc on replicating T-helper cells.

Therefore , one can safely conclude that higher zinc level over the established requirement in corn-soy bean poultry diet can improve HI titres as well as protection percentage on against ND virus.

Table (6) showed the relative weight of some lymphoid organs that partially played an important role in body resistance. The data revealed no consistent pattern for their weights. However, one can notice that best relative weights were recorded in group of chicks (4) which received sufficient zinc levels followed by group 3 that received higher supplemental

Table (6): Relative weight of some lymphoid organs in different groups of chicks fed various levels of zinc.

Age in weeks	Group No.	Weight of spleen	Weight of ceacal tonsile	Weight of Harderian gland	Weight of bursa
4	1	0.23	0.029	0.029	-
	2	0.065	0.065	0.065	0.196
	3	0.171	0.068	0.034	0.239
	4	0.168	0.056	0.056	0.23
5	1	0.014	0.042	0.02	0.045
	2	0.144	0.042	0.026	0.132
	3	0.198	0.105	0.042	0.197
	4	0.245	0.066	0.035	0.197
6	1	0.097	0.19	0.017	0.064
	2	0.082	0.32	0.026	0.179
	3	0.161	0.54	0.045	0.289
	4	0.399	0.63	0.043	0.283
7	1	0.088	0.034	-	.065
	2	0.103	0.053	0.22	0.123
	3	0.164	0.071	0.39	0.138
	4	0.122	0.082	0.07	0.144
8	1	-	-	-	-
	2	0.194	0.126	0.088	0.14
	3	0.286	0.154	0.105	0.188
	4	0.411	0.19	0.146	0.236

zinc, while chicks of group 2 which were fed low available zinc diet recorded the lowest values especially for spleen and bursa of Fabricius.

The obtained results could be confirmed by Barn's work in (1983), who observed that bursa of Fabricius was the most sensitive organ that showed a major localization of immunoglobulins in birds.

SUMMARY

Two hundred and twenty one-day old Hubbard broiler chicks were used to study the possible role of dietary zinc on chicks performance in relation to Newcastle disease vaccine, challenge and relative weights of some immune lymphoid organs. Feeding different levels of supplemental zinc 50 and 150 mg/kg as zinc oxid had significantly improved 8-weeks body weight, feed efficiency, HI titres against ND virus as well as protection percentage and relative weights of spleen and bursa of Fabricius.

On the other hand, increasing the level of calcium in the corn soybean meal diet without any zinc supplement was associated with zinc deficiency symptoms as well as lowered body weight, HI titres and protection percentage against ND virus.

The present study supports that corn soybean meal diet cannot cover the zinc requirements for broiler, An interesting notice, which needs further investigation, that zinc supplement to such diet play an important role as an immuno-stimulants.

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