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Influence of Feeding Zinc Additives on Chickens Blood Components of Immunological Properties

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ONE-YEAR old Dokki cocks were used to study the effect of different levels of dietary zinc on some blood components connected with immunological properties. Cocks were divided into three groups. Group A was considered as a control and was fed a basal ration (B.R.) contained 19 mg Zn/Kg. Group B was fed B.R. + 25 mg Zn/Kg diet. The third group (C), was fed B.R. + 50 mg/Kg.

In groups including zinc-supplemented birds, there was a tendency of increase in leukocytes number and level of zinc in whole blood, though it was not statistically significant. Total serum protein, percentage of eosinophils, basophils and monocytes were not affected. However, lymphocytes percentage was increased and neutrophils percentage was decreased, both significantly in groups which received zinc additives.

Cocks were injected with sheep red blood cells as antignes. Levels of agglutinating antibodies in sera from different groups were not different. However, the significant increase in lymphocyte populations of cocks which have received zinc supplements indicates a possible increase in their immunological capability.

Extensive physiological and nutritional studies on poultry has shown that zinc is an essential trace element for optimal growth (O'Dell *et al*, 1958 and Supplee *et al*, 1958), development (Blamberg *et al*, 1960), and reproduction (Kienholz *et al*, 1961).

Zinc was shown to be present in the prosthetic group of the enzymes: carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase and in others (Keilin and Mann, 1939, Vallee and Hoch, 1957, Matheis, 1958 and Li (1966).

Under normal conditions, zinc appears to be a constant constituent of plasma, erythrocytes and leukocytes. Leukocytes contain 25 times as much zinc per cell as do erythrocytes (Vallee and Gibson, 1949). Human plasma contains about 120mcg Zn/100ml, about 34 % of it is firmly bound to globulins, the remaining is more loosely bound to plasma proteins and probably represents the transport fraction (Wohl and Goodhart, 1964).

Differences in total serum proteins (Iuecke *et al.*, 1958; Rahman *et al.*, 1961 and Fox & Harrison, 1965) and in plasma protein patterns particularly in the gamma globulin and albumin fractions (Moefer *et al.*, 1960; Ott *et al.*, 1965; Makarova, 1965 and Glooshkova, 1966) were observed to be associated with zinc level in diets. Moreover, Miller *et al.* (1968) suggested a diminution in ability of the zinc-deficient animal to utilize globulins and perhaps a diminution in its ability to synthesize serum albumin.

Observations that addition of zinc to the diet increased the blood leukocytes population, specially lymphocyte population, were reported by Mitsik, (1964) ; Glooshkova (1966) and Miller *et al.* (1968).

The previous findings have led to the hypothesis that the zinc may affect the immunological characteristics of chicken's blood. The experiment reported in this paper was set up to ascertain whether zinc supplementation to practical chickens' rations, would affect some blood components connected with immunological properties, namely white blood cell (WBC) number, differential count of WBC, serum protein percent and level of agglutination antibodies (agglutinins).

Material and Methods

1. *Chickens.*

Twenty one Dokki-4 cocks, one year old raised at Assiut University Poultry Farm were randomly designated for this study. Dokki-4 chickens were developed as a new breed from a cross between the indigenous Fayoumi and the Barred plymouth Rock (Itriby and Sayed, 1966). Seven cocks were assigned to each of three groups (A, B and C) considering the initial weights to be as close as possible in all groups . Birds were kept individually in galvanized cages and supplied with feed and water ad-libitum, both in galvanized pans.

2- *Scheme of the experiment*

All groups were fed a basal ration (Which contained 19 mg Zn/kg) during the 15th days pre-treatment and the 30 th days post-treatment periods. Group A was considered as a control and was fed the basal ration during the treatment period which lasted for 45 days. The basal ration was supplemented during the treatment period with 25 mg Zn/Kg for group B, and with 50 mg Zn/Kg for group C. Zinc was supplemented to the ration in form of zinc sulphate ($Zn\ SO_4.\ 7H_2O$).

The basal diet composition was as follows

Ingredient	%
Yellow corn	25
Sorghum (Milo)	30
Decorticated cottonseed meal	20
Wheat bran	20
Blood meal	2
Limestone	3
Common salt (sodium chloride)	0.5
Vitamin premix (A + D_3)*	0.1
Calculated analysis : **	
Crude protein	18.2
Metabolizable energy, Kcal/Kg	2640
Calcium Percent	1.22
Phosphorus percent	0.61

* Vitamin premix contained : V.A. 5000 I.U. and VD_3 500 I.U. per gram.

** Calculated analysis : Values were calculated according to Ewing (1963).

3. *Determinations of blood components*

At the end of the treatment period, blood sample from each bird was taken from the wing vein. A count of the total white blood cells was carried out using a mixture of Giemsa and methyl violet dyes as a diluting fluid. From each bird, three blood films stained with leishman dye were used for differential count of WBC. Total serum protein was determined colorimetrically (Petroonkina, 1961) using Vinkler solution (potassium iodide + mercuric chloride). Also, zinc content of whole blood and of the basal ration were determined colorimetrically (Tawtsin, 1968) after extraction with dithizone (diphenyl thiocarbezone). Statistical analysis including analysis of variances and comparisons of means using Duncan's test were carried out as outlined in Steel and Torrie (1960).

4. *Testing Sera for agglutinins*

Every cock received only one intravenous injection of sheep red blood cells (SRBS) two days after the treatment period. Doseges of the antigen were injected according to the cocks' body weights (3 ml. of 30% SREC suspension in saline/Kg body weight). Sera were individually tested for the presence of agglutinins to SRBC antigens prior to immunization and twice a week for the next month after immunization. The standard procedure for titering serum samples was carried out as outlined by Fanguy (1961) using suspensions of three percent SRBC.

Results and Discussion

a. *Blood Values*

The results obtained from analysis of blood samples are summarized in Table I. Arithmetic means of different groups were statistically compared using Duncans 'test (Tables 2, 3). No significant differences were found between the different groups regarding to leukocytes number, total serum protein percentage and zinc content of whole blood ($F < 0.05$). However, there was a tendency of increase in WEC number in the zinc-supplemented birds, as it ranged from 19,866 to 26,893/mm^3 in case of group A and B respectively. The high variability within each group may explain why such differences between groups were not significant.

Leukocytes have important immunological function involving phagocytosis of microorganisms and foreign particles, antibody production and removal of toxins (Babsky *et al*, 1970). Both Mitsik (1964) and Glooshkova (1966) have reported that zinc addition to the ration increased WEC in cattle. Our findings are analogous to those of Miller *et al*, (1968) in baby pigs.

It can be noticed from Table I that total serum protein had slightly decreased with increasing zinc in the diet as it ranged from 6.54 to 6.76% for group Ç and A, respectively. A decline in plasma protein levels has been observed in zinc deficiency in some investigation (Ott *et al*, 1964 and Rahman *et al*, 1961). On the other hand ,Ott *et al*, (1965) and Miller *et al*, (1968) reported a decrese in serum protein in zinc-supplemented animals. As shown in Table I, whole lood contained 443, 516, and 461 mcg Zn/100 ml in case of group A,

TABLE I. Blood values in Dokki cocks raised on rations enriched with zinc

Blood values Groups	WBC number (Thousands/mm³)	Total serum protein (g %)	Zinc content of whole blood (μ g/100 ml)	Differential count of WBE				
				Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
A	19.866 ± 1.551	6.76 ± 0.60	443 ± 45	15.9 ± 0.6	5.4	0.7	76.0 ± 1.2	2.0
B	25.893 ± 2.592	6.68 ± 0.44	516 ± 32	11.4 ± 1.7	3.5	0.3	83.9 ± 1.8	0.9
C	25.670 ± 2.637	6.54 ± 0.44	465 ± 47	7.5 ± 0.8	6.0	0.3	85.5 ± 1.7	0.7

TABLE 2. ANOVA for blood values in Dokki cocks

Source of variance	D.F.	Mean squares				
		WBC	Total Serum protein	Zn in whole blood	Neutrophils %	Lymphocytes %
Treatments	2	81.725.856	0.0331	9.720.5	124.4	178.3
Error . . .	18	37.550.558	1.7474	12.377.6	9.0	17.2

TABLE 3. Duncan's multiple range test for significant differences among all possible comparisons*

Character	Groups means		
	A	C	B
WBC	19.866	25.670	25.893
	C	B	A
Total serum protein	6.54	6.68	6.76
	A	C	B
Zinc in whole blood	443	465	516
	C	B	A
Neutrophils %	7.5	11.4	15.9
	A	B	C
Lymphocytes %	76.0	83.9	85.5

* Any two means not underscored by the same line are significantly different at 0.05 level.

B and C, respectively. Our data concerning the non-significant increase in zinc content of whole blood with dietary zinc elevation coincide with those of Miller *et al.*, (1962) and Turk (1965) who reported that dietary zinc level had no effect on the zinc content of the blood or the plasma of claves and chickens. Different results have been observed in pigs (Miller *et al*, 1968).

Although blood leukocyte population did not significantly increase with zinc supplementation to the ration, yet a relative increment in lymphocyte population and a relative reduction in neutrophils were observed with zinc additions ($P>0.05$). These results confirm those of Nicimoora and Koomasera (1958)and Miller *et al*, (1968). Other leukocyte types percentages, namely eosinophils, basophils and monocytes percentages were not significantly affected ($P< 0.05$).

The high variability in some blood components within each group is expected since the experimental birds were not genetically homogenious and because, of the nature of characters studied. Comparing data of different experiments, Lucas and jamros (1961) emphasized the high variability in leukocytes formula among different groups of chickens.

Lymphocytes are known to be the most elastic of all the blood cells. They may transform to macrophages, monocytes, plasma cells and other hemocytes which are connected with antibody production and mediated cellular defence reactions (Daniels *et al*, 1968).

The increase in Lymphocytes number in treated groups may be due to the indirect effect of Zn on the central lymphoid system as thymus gland. Thymus weight and its content of Zn were greatly reduced in Zn-deficient animals (Shanklin *et al*, 1968).

b. *Formation of antibodies to sheep red blood cells*

Serum samples titers were obtained by making serial double dilutions in isotonic saline solution. The highest dilutions at which antisera showed visible macroscopic reactions with SRBC were recorded. Range of titers for every group of cocks' sera and their means are shown in Table 4.

Sera collected from cocks prior to immunization showed no agglutination with SRBC. However, two serum samples, one from B group and the other from C group, caused lysing of SRBC up to 1: 8 delutions. Such observation suggest the presence of haemolysin antibodies, but not agglutinins, to SRBC in normal sera of some cocks.

As shown in Table 4, agglutinins to SRBC were detected in all groups by the 4*th* day after immunization, increased by the 7*th* day and gradually declined thereafter. Comparing the titers means of the three groups, there are no special differences at all periods tested. However, the highest agglutinins titer (1 : 2048) was obtained from antisera of some individuals in group B and C which recieved Zn additives. Haemolysis of SRBC was noticed in case of all of all immune sera up to 1 : 4-16 dilutions in all grorups. The presence of haemolysins was noticed even after disappearance of agglutinins. No special differences were found between the individual groups in level of haemolysins.

TABLE 4. The range of titers of agglutinins in cocks' sera to sheep red bood cells and their means expressed as log 2*

Group / Days	A Basal ration (B.R.)		B (B.R.+25 mg Zn/kg)		C (B.R.+50 mg Zn/kg)	
	Range	Mean	Range	Mean	Range	Mean
Pre-injection day	0 — 0	0	0 — 0	0	0 — 0	0
Post-injection day :						
4th	1 : — 128	7.5	1 : 64— 256	8.14	1 : 128— 512	8.57
7th	1 : 1024—1024	11.0	1 : 512—2048	10.7	1 : 512—2048	10.87
10th	1 : 128— 512	8.67	1 : 16— 256	8.0	1 : 16—2048	7.86
14th	1 : 16— 256	7.0	1 : 4— 256	6.43	1 : 8—1024	6.43
17th	1 : 8— 64	4.83	1 : 2— 128	5.28	1 : 8— 128	4.86
21th	1 : 4— 16	3.33	1 : 2— 32	3.56	1 : 14— 16	4.14
24th	0 — 1 : 2	H**	0 — 1 : 32	H	0 — 1 : 2	H
28th	0 — 0	H	0 — 1 : 8	H	0 — 0	H

* Positive reaction at dilutions of 1 : 2, 1 : 4, 1 : 8 was transformed to 1, 2, 3, respectively and so forth.

** Haemolysis of sheep red blood cells.

In conclusion, no substaintial increase was found in antibodies production in cocks receiving Zn additives, which indicates that 19 P.P.m. Zn present in basal ration may be sufficient. Zinc requirements of chicken are not well defined as they ranged from 15 to 20 mg as reported by Zeigler *et al*, (1961) and from 50 to 60 mg/Kg diet as reported by Mitsik (1964). However, the increase in lymphocytes percent of cocks received Zn supplementations may suggest an increase in their immunological capability. Supplementing chicken rations with Zn may have more pronounced effcet at younger ages before they are immunologically mature.

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تأثير اضافة الزنك الى العليقة على بعض الصفات المناعية في دم الكتاكيت

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استخدمت ديوك دقى عمر عام لدراسة تأثير المستويات المختلفة من الزنك فى العليقة على بعض الصفات المناعية فى دم الطيور .

قسمت الطيور الى ثلاثة مجاميع : أعطيت مجموعة المقارنة (أ) العليقة الأساسية وهى تحتوى ١٩ ملليجرام زنك / كجم عليقة بينما أعطيت المجموعة الثانية (ب) اضافة قدرها ٢٥ ملليجرام زنك / كجم عليقة وأعطيت المجموعة الثالثة (جـ) اضافة قدرها ٥٠ ملليجرام زنك / كجم عليقة . واستمرت مدة المعاملة ٤٥ يوما .

وقد اتضح بدراسة دم الطيور أن هناك ميلا للزيادة فى عدد كرات الدم البيضاء وفى مستوى الزنك فى الدم فى المجاميع ب ، جـ وان كانت الزيادة غير معنوية . بينما لم تتأثر نسبة البروتين فى الدم او النسبة المئوية لكل من الايوسينوفيل والبازوفيل والمونوسيت . وقد وجدت زيادة معنوية فى نسبة اللمفوسيت ونقصا معنويا فى نسبة النيوتروفيل فى المجاميع التى تناولت الاضافات من الزنك .

ولم تلاحظ أى فروق معنوية بين المجاميع الثلاثة بالنسبة لانتاج الأجسام المضادة عند حقن الطيور بالكرات الحمراء لدم اغنام . واعتمادا على زيادة نسبة اللمفوسيت فى المجاميع التى تناولت كميات اضافية من الزنك يمكن القول بأن ذلك قد يزيد من القدرة المناعية للديوك .