Effect Of Copper As Feed Additive On Body Weight, Feed Efficiency And Immune Response Of Broilers

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ABSTRACT

This work was carried out to study the effect of different levels of copper supplementation in the broilers diet on growth performance and immune response. It was conducted with one hundred and twenty, one day-old Cobb broiler chicks that were evenly distributed to four equal groups of 30 chicks for each. They were fed on four experimental rations for six weeks experimental period. The first group was fed on a basal diet (control group ,G1), and the remaining groups were fed 63(G2), 75 (G3), or 125(G4), mg Cu /kg diet.

Body weight and feed efficiency were determined weekly. Also the primary and secondary antibody responses were determined. When the chicks were 4wk of age, a delayed type hypersensitivity test was performed. White blood cells were differentiated and antibody response was determined.

The body weight significantly increased in G2& G3. However, statistical analysis revealed non-significant differences between G3, G4 and control groups.

Concerning feed efficiency 125ppm Cu additive increased FE while 63 and 75ppm Cu reduced it. Regarding the effect of Cu on the immune response there were a significant increase in the ratio of bursa of Fabricius weight indecies, and increased lymphocytic counts. However, all levels of Cu increased haemagglutenin antibody titers, cell mediated responses to phytohemagglutitn, non-significant increase in thymus indecies and reduced Heterophil/Lymphocyte ratios. In conclusion the present study showed that all dietary Cu levels had improved the cellular and humeral immune response, while 63 ppm Cu increased the live body weight and 125 ppm Cu increased feed efficiency.

INTRODUCTION

Copper has been hypothesized as playing an important role in the defense of the organism against metabolic, neoplastic, infectious, inflammatory and other forms of stresses. Therefore, copper deficient animals show decrease in antibody cell response with increased susceptibility to infection *(1)*. Copper (Cu) is an essential micro-mineral that can be added to broiler chicken diets at high levels, acting as a growth promoter *(2)*.

The immune system of the chicken is the corner stone in preventing diseases, and in order to insure maximum productive potential it is very valuable to know how to take advantage of all parts of the system when designing health and therapeutic program *(3)*.

MATERIAL AND METHODS

The objective of this study is to investigate the effect of different levels of copper as copper citrate, on weight gain, feed efficiency and immunity of broilers. One hundred and twenty day-old commercial (Cobb) broiler chicks randomly distributed into four equal groups, each group contained 30 chicks, and has free access to experimental feed and water all times, and each group was treated nutritionally as G1 (Control), G2 (63mg Cu/kg diet), G_3(75 mg Cu /kg diet), G_4(125 mg Cu /kg diet) all birds in different groups are fed from one day to 42 days of age as mentioned by ***(4 and 5)***. The chicks were reared under hygienic condition in battery system, and fed on diets formulated to meet the nutritional requirements as suggested by the *(6)*, Table (1).

Cu was added as copper (II) citrate 2.5 hydrate ($C_6H_4O_7CU_2.2.5H_2O$) = 360, Wako pure chemicals industries, Ltd. The percentage of the copper within the copper citrate was calculated to reach the required dietary tested

level in this study. Birds were weighed and feed efficiency (FE) was calculated weekly according to the equation, FE=Gain produced in a given period/Feed consumption of the same period

Immune response was evaluated by: (1) Cellular immune response: At 40 days of age blood samples were directly prepared from each group in order to determine total leukocytic count according to the method described by *(7)*, and then blood films were prepared and stained with Giemsa stain for deferential leukocytic count. One hundred cells were counted and differentiated into Heterophiles (H), Lymphocytes (L), Basophiles and eosinophiles. The mean H / L ratio was calculated from individual H / L ratio *(8)*.(2) Inter digital skin test: It is a simple skin test to evaluate cell mediated immuno-competence in chicken at the 4th week of age by in vivo stimulation with a non specific mitogen phytohem- agglutinin (PHA) ***(9 &10)***. (3) Lymphoid organ weight index: According to *(11)* spleen, bursa of Fabricius and thymus gland of chicks were removed, cleansed of adhering material and weighed at 1st & 2nd blood sampling at 26 & 40 days of age respectively. The organs weight was expressed as percentage of live body weight, and calculated as follow, [organ weight (g) / body weight (kg)] ×100. (4) Humeral immune response: Carried through Haemagglutination Inhibition test. All antibody titers were recorded of the highest dilution of serum that agglutinated on equal volumes of 0.05% sheep red blood cells (SRBCs) suspension in phosphate buffered saline (PBS) *(8)*.

Copper was determined spectrophotometerically according to *(12)*. All obtained data were statistically analyzed according to ***(13)***.

RESULTS AND DISCUSSION

Results showed significant increase (P<0.01) and (P<0.05) in live body weight in G2 &G3 respectively when compared with control group Table (2).

These are greatly similar to findings stated by *(4, 14-16)* who mentioned that copper citrate significantly increased broilers growth at lower levels than copper sulfate that may be due to better absorption of copper citrate than copper sulfate. Results was indicated a non significant increase in live body weight of 125 ppm copper treated group when compared with control one. These results in agreement with *(15,17)* who indicated that copper from copper citrate has no effect on growth response in broilers chicks, but in contrast to findings of *(4)* who mentioned that 125 mg copper citrate per Kg diet for 42 days significantly increased broilers growth.

Feed efficiency was listed in Table (3), and revealed that there was a slightly improvement in feed efficiency in G4. These results were in accordance with *(18)* who stated that supplementation of low copper (125 mg per Kg diet) gave better performance than the supplementation of 250 mg copper sulphate per Kg diet in broiler chicks.

Regarding the immune response (Table 4), showed that spleen weight indecies was increased significantly (P<0.05) in G2 and highly significant (P<0.01) in G3 where there was non-significant increase in group G4 when compared with control group at the second blood sample. Also we noticed that significant increase (P<0.01) of bursa weight indecies in G2 & G3, where there was non-significant increase of bursa weight indecies in G4 when compared with G1 at the second blood sample that support the enhancing effect of copper on humeral immune response. Moreover, thymus weight indecies were showed a non-significant increase with increased dietary copper additives when compared with control group at both first and second blood samples. These results were in accordance with ***(19,20)***, who mentioned that copper function in immune system through, energy production, neutrophil production, antioxidant enzyme production, development of antibodies and lymphocyte replication. Mean levels of haemagglutenin antibody titers in chicks immunized with sheep RBCs showed in Table (5), and revealed that non-significant increase in antibody titer levels when copper increased at the first immunization, but at the second immunization

(Challenge) we noticed that significant increase ($P<0.05$) of antibody titers in G3 & G4, these results incorporate with high bursa weight indecies that support the enhancing effect of copper on immunity.

In this study and according to Table (6) no effect of any level of dietary copper additives on WBCs counts but the H/L ratios were reduced. The increased lymphocyte counts and the decrease in H/L ratio in all Cu-treated groups may suggest that organic copper improved specific immune response ***(19,20)***. Moreover, the inflammatory response to mitogen (PHA) was evaluated and showed in Table (7). The higher response of copper treated chicks to PHA than the control group may indicate an improvement of cell-mediated immunity that coincides with results of ***(21)*** who found that copper deficiency can numbers of cells mediating immunity.

Table 1. The composition and calculated chemical analysis of the experimental diet

Ingredients	Starter(0-3weeks)	Grower(4-6weeks)
Ground yellow corn	56.7	66.6
Soya bean meal 44%	29.5	23.53
Fish meal (magrabi) 60.5%CP	7.0	5.0
Soya bean oil	4.06	2.02
Dicalcium phosphate	0.88	0.6
Limestone	1.26	1.69
DL – Methionine (96%)	0.1	0.06
Iodized sodium chloride	0.25	0.25
Vitamins & mineral premix*	0.25	0.25
Calculated composition		
Crude protein	22.0	19.0
ME kcal per kg diet	3060.0	3040.0
Calorie/protein ratio(C/P)	139.0	160.0
Copper (ppm)	9.12	8.3

* Each 2.5 kg contains vitamins and minerals premix as follow:
Vit. A 12 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 2000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1600mg, vit. B_{12} 10mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 6000mg, zinc 5000mg, iron 3000mg, copper 10000mg, iodine 1000mg, selenium 100mg, cobalt 100mg, carrier($CaCO_3$) to 2.5kg. (AGRI-VET. Under technical assistance of HELM Germany)

Table 2. Average live body weight (g) in groups fed supranormal levels of copper additives.

Group / Age/wk	Control	Copper(63ppm	Copper(75ppm)	Copper(125ppm)	LSD 0.05	LSD 0.01
Initial Wt.(g)	40.4±0.5	40.4±0.5	41.2±0.7	40.0±0.5	No sig.	No sig.
1	121.3±1.98^{a}	123.1±2.1^{a}	114.96±1.9^{b}	117.9±2.2^{b}	4.95	7.2
2	352.0±4.8^{a}	352.3±6.4^{a}	329±6.3^{b}	346.7±5.3^{a}	13.8	20.0
3	684.9±8.2	721.2±9.2	695±11.7	700.1±9.0	No sig.	No sig.
4	1082.7±12.6^{bc}	1177.8±6.9^{a}	1116.1±14^{b}	1073.2±20.3^{c}	34.55	50.3
5	1445.9±23.6^{b}	1622.4±19.5^{a}	1600.3±28.7^{a}	1499.3±22.5^{b}	57.57	83.86
6	1881.5±30.2^{b}	2039.2±35.3^{a}	1966.6±35.4^{a}	1923.1±39^{ab}	85	123.7

Values are means ± SE a,b,c,…etc: means within the same row with different superscripts are significantly different.

Table 3. Influence of different levels of dietary copper additives on feed efficiency

Groups / Age /w	G1	G2	G3)	G4
0-1	0.83	0.91	0.81	0.89
1-2	0.80	0.66	0.72	0.75
2-3	0.69	0.68	0.70	0.76
3-4	0.62	0.59	0.63	0.60
4-5	0.53	0.53	0.65	0.67
5-6	0.50	0.40	0.39	0.43
Total	0.60	0.55	0.59	0.61

Table 4. Effect of different levels of dietary copper additives on lymphoid organs weight index*.

Organ / Group		Control	Copper (63ppm)	Copper (75ppm)	Copper (125ppm)	LSD 0.05	LSD 0.01
Spleen	1st blood sample	1.05±0.22	1.13±0.04	1.04±0.31	1.03±0.18	No sig.	No sig.
	2nd blood sample	0.76±0.1^{c}	1±0.03^{b}	1.3±0.05^{a}	0.8±0.08^{c}	0.2	0.3
Bursa	1st blood sample	1.3±0.2	2.0±0.1	1.7±0.5	2.0±0.2	No sig.	No sig.
	2nd blood sample	0.5±0.02^{c}	0.8±0.07^{b}	0.99±0.1^{a}	0.65±0.06^{b,c}	0.18	0.28
Thymus	1st blood sample	2.3±0.1	3.0±0.4	3.2±0.5	3.5±0.3	No. sig.	No sig.
	2nd blood sample	1.44±0.2	1.76±0.9	2.3±0.4	2.07±0.2	No sig.	No sig.

Values are means ± SE

a,b,c,....etc: means within the same row with different superscripts are significantly different.

*[Wt (g)/LBWt (kg) ×100].

Table 5. Effect of different levels of dietary copper additives on hemagglutinin antibody titer in chicks immunized with sheep RBCs.

Parameters / Groups		Control	Copper (63ppm)	Copper (75ppm)	Copper (125ppm)	LSD 0.05	LSD 0.01
Haemagglutinin antibody titer	1st blood sample	3.7±0.3	4.3±0.3	4.7±0.7	5.3±0.3	No sig.	No sig.
	2nd blood sample	4.3±0.3^{b}	5±0.6^{ab}	7.3±0.3^{a}	6.3±0.9^{a}	1.5	2.4

Values are means ± SE

a,b,c,....etc: means within the same row with different superscripts are significantly different.

Table 6. Mean values of white blood cells (WBCs) count in groups fed different levels of dietary copper additives.

Parameter \ Group		Control	Copper (63ppm)	Copper (75ppm)	Copper (125ppm)	LSD 0.05	LSD 0.01
Total. L.C /ul		21979.0±800	22389.0±702	21341.0±696	22722.0±563	No. sig.	No. sig.
Diff. L.C	Monocyte	4.0	5.0	6.0	6.0		
	Basophile	1.0	0.0	0.0	0.0		
	Eosinophile	3.0	4.0	2.0	3.0		
	Heterophiles	32.0	30.0	29.0	30.0		
	lymphocyte	60.0	61.0	63.0	60.0		
	H/L ratio	0.53	0.49	0.46	0.50		

Values are means ± (SE)

$^{a,b,c,...etc:}$ means within the same row with different superscripts are significantly different.

Table 7. Effect of copper supplementation on skin response of broiler to PHA and PBS (thickness in mm):

Hours Post	Control		Copper (63ppm)		Copper (75ppm)		Copper (125ppm)		LSD 0.05
	PBS	PHA	PBS	PHA	PBS	PHA	PBS	PHA	
0	1.04±.11	1.07±.08	1.23±.02	1.26±.01	1.13±.03	1.17±.03	1.14±.01	1.2±.06	No sig.
6	$1.33±.01^{bc}$	$1.39±.02^{a}$	$1.36±.01^{a}$	$1.38±.01^{a}$	$1.31±.01^{c}$	$1.39±.01^{a}$	$1.34±.01^{ab}$	$1.37±.01^{a}$	0.04
12	$1.34±.01^{c}$	$1.38±.01^{ab}$	1.32±.01	$1.39±.01^{a}$	1.34±.01	$1.41±.01^{a}$	$1.35±.01^{c}$	$1.37±.01^{bc}$	0.03
18	$1.4±.01^{b}$	$1.4±.01^{b}$	$1.39±.01^{bc}$	$1.42±.02^{ab}$	$1.36±.02^{c}$	$1.43±.01^{a}$	$1.36±.02^{c}$	$1.45±.01^{a}$	0.03
24	$1.33±.01^{e}$	$1.37±.01^{d}$	$1.42±.01^{c}$	$1.45±.01^{b}$	$1.45±.01^{b}$	$1.48±.01^{a}$	$1.45±.01^{b}$	$1.5±.02^{a}$	0.03

Values are means ± (SE)

$^{a,b,c,...etc}$: means within the same row with different superscripts are significantly differ at ($P<0.05$).

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الملخص العربي

تأثير النحاس كإضافة أعلاف علي النمو وكفاءة الغذاء والاستجابة المناعية في بداري التسمين

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أجريت هذه الدراسة علي عدد مائة وعشرون كتكوت عمر يوم من نوع "كب" لمعرفة تأثير إضافة مستويات مختلفة من عنصر النحاس العضوي علي هيئة سترات النحاس علي الزيادة في الوزن إسبوعيا وكفاءة الغذاء وكذلك المناعة في بداري التسمين وقد قسمت هذه الكتاكيت عشوائيا إلي أربعة مجموعات غذيت علي علائق مختلفة لمدة ٤٢ يوم ,المجموعة الأولي وهي المجموعة الضابطه غذيت علي عليقة لاتحتوى علي أى إضافات أما المجموعة الثانية والثالثة والرابعة فقد غذيت علي علائق تحتوى علي ٦٣ ,٧٥ ,١٢٥ مجم نحاس / كجم علف علي التوالي. وقد أثبتت التجربة أن التغذية علي ٦٣، ٧٥ مجم نحاس / كجم علف أدت الي زيادة معنويه في الوزن النهائي ومعدل النمو. بينما أظهرت التحاليل الاحصائية عدم وجود فروق معنوية بين إضافة ٧٥ و ١٢٥ مجم نحاس/كجم علف وبين المجموعة الضابطة و زادت كفاءة الغذاء في المجموعة التي غذيت ١٢٥ مجم نحاس/كجم بينما قلت في باقي المعاملات. لوحظ أيضا أن جميع الكميات المضافة من سترات النحاس أدت إلي زيادة دلائل وزن غدة فابريسي ولم تؤدي الي أي زيادة معنوية في دلائل وزن الغدة التيموسية، وأيضا أدت الي تقليل النسبة بين الخلايا البيضاء المغايرة الي الخلايا الليمفاوية، كما أدت الي زيادة عدد الخلايا الليمفاوية و مستوي الأجسام المضادة لخلايا دم الاغنام الحمراء. كذلك جميع مستويات النحاس المضافة أدت الي استجابة المناعة الخلوية للملزن النباتي للدم.