TOXICITY OF Copper AND Cobalt IN CHICKEN (*GALLUS GALLUS DOMESTICS* ASSESSMENT OF BODY WEIGHT AND METAL CONTENT IN TISSUES AFTER METAL DIETARY SUPPLEMENTS

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ABSTRACT

The toxic influence of dietary copper(Cu) and cobalt (Co) on the accumulation and body weight examined in broiler chicken. This experiment was conducted to investigate the toxic effects of dietary Cu and Co on chicks body weight and organ, content of the tissues of these two metals was also detected. One day age chicks of *Gallus gallus domesticus* fed diet supplemented with 500, 1000,1500ppm of Cu, second group exposure to 100,500, 1000ppm of Co in feed daily during 4 weeks. The control groups were fed without supplementation of metals. The concentrations of Cu and Co resulted in increased of Cu and Co content in liver, gizzard and muscle. Body weight of chicks was decreased by Cu and Co high concentration treatments. On the other hand, Liver weigh in chicks was significantly ($P<0.05$) decreased after Cu and Co treatments.

.INTRODUCTION

Copper is an essential trace element that is particularly toxic to organisms and organs at high doses. It is a fairly plentiful trace element that is important for numerous metabolic processes and enzyme systems (1). Copper is essential in bone formation, skeletal mineralization, and maintaining the integrity of connective tissues. (2) reported that high intake of copper can cause health problems, such as liver and kidney damage. High concentration of copper can also cause public health hazards (3) .Long-term exposure to copper can cause irritation of the nose, mouth, and eyes, as well as headaches, stomachaches, dizziness, vomiting, and diarrhea. Purposely high uptakes of copper may cause liver and kidney damage and even death in human. However, whether copper is carcinogenic has not been determined yet. Higher concentration of Cu in the diet depresses growth and feed efficiency in chicken and cusses damages both gizzards and liver function (4),(5) . A high dietary copper supplementation of 500 mg/kg did significantly depress growth and feed conversion in the chicken (6) . Chen *et al.* (1996) observed an increase in the enzymes level as a result of increase in the level of dietary Cu in country chicken and pullet chickens.

Cobalt occurs naturally in soil, rock, air, water, plants, and animals. Cobalt is favorable for humans because it is part of vitamin B12; thus essential for human health. Cobalt is used to treat anemia for pregnant women because it stimulates the production of red blood cells. The total daily intake of cobalt varies and may be as much as 1 mg (7). The US National Research Council (NRC) set 25 mg/kg feed as the maximum tolerable level of cobalt for cattle, poultry, sheep and horses, and 100 mg/kg feed for swine (8).

In birds, Diaz *et al* (9) demonstrate that excessive dietary cobalt has serious adverse effects on the health and performance of broiler chickens. They found Dietary levels of 0, 125, 250, and 500 mg of cobalt per kg of feed were reduced feed intake, body weight gain, and gain: feed ratio and caused a dose-dependent increase in mortality .On the other hand, Chickens fed 250 and 500 mg cobalt/kg feed developed pancreatic fibrosis, multifocal hepatic necrosis, and lesions in skeletal and cardiac muscle and smooth muscle of the duodenum. Cobalt was decreased by increased dietary protein as measured by the interactions on growth (10) . (11) found the growth of chick revealed a significant cobalt-protein interaction indicative of the fact that cobalt was less toxic when fed with higher levels of protein. Normal levels of cobalt in poultry diets are 0.1 to 0.5 parts/106 and the element is mainly present as part of the vitamin B12which is normally present in corn and soya bean. Cobalt will accumulate in plants and in animal bodies that eat these plants. However, cobalt is not known to bio magnify up the food chain because the fruits, vegetables, fish, and other animals that we eat do not usually contain high amounts of cobalt. Cobalt has been reported to induce cardiomyopathy in man and in animal species including swine, dogs, and rats (12). The aim of present study to determinate of the exposure to Cu and Co during the development period which includes chicks body weight and tissues content of heavy metals. Few studies aiming at enhanced understanding of the impact of heavy metals in birds have been demeanor.

MATERIAL AND METHODS

Animals and Experimental Design

Gallus domesticus fertile eggs were obtained from a commercial (poultry farm, Malaysia). The eggs were cleaned, labeled and weighed 57.537±7.817 g, rang 51-65 g. Then put in incubator. After 21 days, eggs hatchling, one day age chicks were randomly assigned into seven groups (n=10 each) according to dietary copper and cobalt. (1) control, (2) Cu 500ppm, (3) Cu 1000 ppm, (4) Cu1500ppm, (5) Co 100ppm, (6) Co 500ppm and (7) Co 1000ppm. The animals were housed for 4 weeks in groups in stainless steel cages home (90 cm×60cm×50 cm) with warmth provided by OSRAM lamps (100 W). Drinking water and commercial diets were offered ad libitum

The chicks were fed starter diet to 3 week of age and a grower diet until the end of experiment(Table 1), The toxic concentration of metals(Co and Cu) was chosen depend on Nutrient Requirement of Poultry by National Research Council (NRC) (13). The first treatment groups were fed the experimental diets which consisted of the basal diet supplemented with 500, 1000, and 1500 ppm of Cu as (CuCl2. H2O). While the second groups were fed the food supplemented with 100,500, and 1000 ppm Co as (CoCl2.6H2O). Dietary supplements to control group without addition of metals .Body-weight and data were obtained bi-weekly. At the end of the 4-week exposure period, the chicks were weighed and sacrificed, and the liver, gizzard, heart and feather were removed and samples of liver, gizzard breast muscle and feather were collected and stored prior to analysis.

Table 1 Composition of poultry feed (starter and grower)#

Specification	Starter	Grower
Crud protein	22.05%(min)	18.03%(min)
Crud fat	3.15%(min)	3.15%(min)
Crud fiber	3.5%(max)	6.4%(max)
Total ach	7.4%(max)	7.4%(max)
moisture	13%(max)	13%(max)
phosphorus	0.60%(min)	0.50%(min)
Calcium	0.9-1.8%	0.9-1.8%
Soybean meal	44%	44%
Vitamin premix	0.5%	0.5%

#NRS,1994

Determination of metal concentration in chick tissues

Sample of liver, gizzard and pectoral muscle were thawed. The samples of feather were washed in de-ionized water, which was alternated with acetone to remove loosely adherent external contaminations. All the tissue samples were dried in an oven at 80ºC for 48 hour or until a constant dry mass was achieved. Then the dryer samples ground using a mortar to powder**.** The feathers were homogenized using electric blender (Philips-HR-1741). The samples weighted about 0.5g of each tissue. The individual tissue samples were digested with 70% nitric acid and 30% hydrogen peroxide (2:1), according to standard method of analytic (14), and left in room temperature overnight. The samples were completely digested in block thermostat (150ºC) for 4 hours until the solutions became clear. After cooling, the solution was made up to 50ml with de-ionized water. Cu and Co concentration determined using inductively coupled plasma-mass spectrometry (ICP-MS, model Perkin-Elmer Elan 9000 A). Quality assurance procedures included the analysis of reagent blanks and appropriate standard reference material for lobster hepatopancreas (TORT-2, National Research Council Canada). The recovery of Cu was 78.44 ±2.11 % and that of Co was 83.92 ± 4.81%.

Statistic analysis

Data were expressed as means ±SD**.** The values were analyzed by Independent sample t- test, was performance to found significant differences between metals concentration in treatment group and control. Differences at $P<0.05$and 0.01 were considered statistically significant.

RESULT

Body weight of the chicks in the control group and the groups treated with metals increased in rapidity from the age of one day to a grown old age of 28 days. Whereas chicks have a lower average body weight weekly throughout the experimental period in four weeks compared with the control group. According to table 2, the chicks weight after 2 week of dietary supplement Cu was decreased significantly ($p<0.05$) in (Group -4) Cu1500ppm compare with control. While dietary Co at concentration 500, 1000, ppm(Group 6&7) did affect significantly the final body weight.

After 4 week dietary Cu supplementation in the diet at 500, 1000, 1500ppm were significantly affect the chicks weight gain compared with control. On the other hand supplementation of either diet with 500 and 1000ppm cobalt had significantly effect on chicks weight and growth of the three groups of treated chicks.

Table 3, showed that the liver, gizzard and heart weights in chicks treated with Cu concentration and in the control treatment. It noted that the chicks treated Cu500ppm,(G2) Cu1000ppm(G3) and 1500ppm (G4)of Cu was significantly (*P* <0.05) in liver weight decreased compared with control treatment. In contrast the weight of gizzard and heart did not affect significantly compare with control treatment. In addition chicks were treated Co concentration seems that the gizzard and heart wear does not affect the concentration of Co while the liver weight was significantly (*P*<0.05) decreased by Co 500 ppm,(G6) and1000ppm.

Table 2 Mean body weight gains (g), after 2 and 4 week of feed supplement metals concentration

Treatment	After 2week	After 4 week
Control	317.2±33.79	635.3±71.32
Cu500	316.3 ± 18.90	461.6 ± 9.19*
Cu1000	267.7 ± 11.71	468.6 ± 6.73**
Cu1500	225.4 ± 7.12**	423.1 ± 5.46**
Co100	275.1 ±51.53	501.6 ± 11.34
Co500	238.5 ± 8.130*	481.0 ± 23.21*
Co 1000	232.5 ± 15.12*	424.0 ± 16.51**

*P<0.05 ANOVA between metal treatment and control
**P<0.01ANOVA between metals treatment and control

Table 3 Organ body weight of chicks affected by nutritional supplement Cu and Co

Organ weight/ g (4 week)			
Treatments	Liver	Gizzard	Heart
Control	24.57 ± 2.314	15.82 ± 0.98	4.42 ± 0.86
Cu500ppm	14.58 ± 1.20*	12.84 ± 0.69	3.90 ± 0.50
Cu1000 ppm	13.72 ± 0.45*	11.48 ± 0.13	2.95 ± 0.32
Cu1500	11.44 ± 1.70*	10.85 ± 0.37	2.52 ± 0.16
Co100 ppm	15.46 ± 1.01*	13.94 ± 0.26	3.39 ± 0.23
Co500 ppm	12.65 ± 0.22**	12.70 ± 0.36	2.87 ± 0.30
Co1000ppm	10.54 ± 0.14**	11.51 ± 0.35	2.75 ± 0.44

*P<0.05 ANOVA between metal treatment and control
**P<0.01ANOVA between metals treatment and control

Bioaccumulation of trace metal in chicks tissues after metals feed supplement

The effect of dietary copper on the copper concentration of chicken selected tissues after 4-week old broiler chickens was studied in the experiment result summarized in table 4. Levels 1500ppm of copper resulted in significant increases in the copper content of liver compare with control. Muscle showed a seriously reduced ability to accumulate copper although the levels of Cu in Cu1500ppm did result in significant increase (9.37µg/g) compare with control treatment. Gizzard accumulated Cu after treatment, and it content from Cu in three Cu groups were not significantly (P>0.05) higher than its level at control. Followed by feather which accumulates high level of Cu at Cu 1500ppmcompare to control**.**

Table 5 showed the effect of dietary cobalt on the tissue cobalt concentration of liver**,** gizzard, muscle and feather of young chicks**.** High level of Co content accumulated in feather than other tissues reached 3.06µg/g. Co content in feather at group Co 1000was higher relatively (P<0.05) compare with control. In contrary, less Co accumulated in pectoral muscle, however, the Co levels in Co 500 and 1000ppm groupwere significantly high than control. Different levels of supplemental Co (100,500,1000ppm) were effect in Co content in liver and gizzard of chicks and Co accumulated in this tissue in higher level compare to control.

Table 4 The effect of dietary Cu on the tissue Cu concentration of liver, gizzard, muscle and feather of young chicks after 4week

	Tissue Cu content (µg/g d.w.) #			
Treatment	Muscle	Liver	Gizzard	Feather
Control ppm	4.826 ± 0.509	28.006 ±0.792	26.33 ±1.813	19.112 ±1.073
Cu 500 ppm	5.180 ± 0.357	25.569 ± 1.070	27.77 ±0.519	21.231 ±1.254
Cu 1000ppm	7.121 ± 0.599	33.450 ± 0.530	28.54 ±2.981	21.812 ±1.342
Cu 1500ppm	9.370 ±0.326*	49.640 ±0.297*	28.98 ±0.353	25.064 ±0.526

* $p<0.05$ independent T-test to compare the difference between Cu treatment and control, # Mean± standard deviation

Table 5 ; Effect of dietary Co on the tissue Co concentration of liver, gizzard, muscle and feather of young chicks after 4 week

	Tissues Co content (µg/g d.w.) #			
Treatment	Muscle	Liver	Gizzard	Feather
Control ppm	0.407 ± 0.019	0.501±0.003	0.344 ±0.021	3.060 ±0.081
Co 100 ppm	0.615 ± 0.052*	0.754 ±0.005*	0.652 ±0.027*	4.410 ±0.039*
Co 500 ppm	0.552 ± 0.024*	0.810 ±0.034*	0.797±0.058*	5.221 ±0.113*
Co 1000 ppm	0.732±0.038*	0.799 ±0.014*	1.723 ±0.133*	5.016±0.610*

* $p<0.05$ independent t-test to compare the difference between Co treatment and control, # Mean± SD.

DISCUSSION

Birds uptake metals from feeds and the surrounding environment (15). Metals can be absorbed through the intestinal tract, circulating through the body and depositing onto different body organs. Metals can also bioaccumulate at different levels to reach toxic levels that can decrease survival and reproductive success in birds (16), (17). Copper can be set such that a metabolically increase in the concentration of metal is not directly proportional to the metal bioavailability, and thus reduces the potential to detect differences in the environmental levels (18). Animals with high levels of liver Cu have relatively low ratios of liver Cd to Cu, which limits any Cd-induced movement of Cu from metallothione in MT. Present result showed that body weight of chick was significantly decreased than control after diet treated with Cu concentration. Same observation was previously recorded by (19) who demonstrated that chicken fed diets supplemented with Cu 350 mg kg-1 supplemented in diet has damaged the gizzard, depressed the feed intake and reduced growth rate of broiler. An addition of 250 ppm copper to the methionine diet appeared to reduce the elevation in spleen or liver of iron concentration. Chicken diet supplemented with high concentration resulted in decreases in 21 body weight , body weight gain and feed consumption(20).On the other hand, present study is contrary to that by (21), which indicated the incorporation of 250 ppm supplemental Cu into the diet resulted in an overall enhance in both body weight gains and feed consumption. (22) reported that addition of Cu concentrations at 450 ppm results in decreased feed intakes against those fed 300 ppm Cu or less. (4) also indicated that elevated concentrations of Cu (500 ppm) resulted in growth depressing effects, but the incorporation of 250 ppm supplemental Cu had no significant effects on body weight gains or feed conversion. Further, poultry chicks treated with copper 250 mg/kg did not effect on performance of broilers, while higher level (500 mg/kg) of copper depress growth , feed conversion in chicken and gastro intestinal morphology (6).Further, (23) was found adding 500 or 750 ppm copper to a diet deficient or just adequate in sulfur amino acids for chicks significantly reduced growth rate and efficiency of food utilization. According to the Nutrient Requirement of Poultry by National Research Council (13) copper as Cu SO3 .5H2Oat 250, 500 and 800 ppm toxic to chicken and reduced growth; gizzard erosion. Lowered body weights in treatment groups birds could be due to reduce in the feed utilization or due to metabolic disarray related with metals, such as inhibition of enzyme involved in the haem synthesis and the oxidase system resulting in loss of cellular functions and tissue damage (24). In Cu supplemented birds, Cu plays a major role as cofactor in hematogenesis (6). Cu is one of the most critical trace elements in livestock because it is necessary for haemoglobin formation, iron absorption from GI-tract and iron mobilization from tissue stores (25) .

Cobalt has more effect on chick weight gain after four week of treatment by 100,500,1000ppm compare to control as we detected in current study. These data are in agreement with (26) has been reported depressed growth rate as the most important sign of excessive cobalt intake in chickens . (11) was mentioned that cobalt has toxicity effect in chicken in levels 50-500 parts/$10^{60}$of cobalt in the diet of chicken . (9) found the effect of high dietary cobalt was a marked reduction in body weight and feed intake compared to birds fed the control diet. These results are in agreement with previous reports that mention a poor performance as the most important sign of excess cobalt intake in broiler chickens (10) and (26). According to the Nutrient Requirement of Poultry by National Research Council (13), cobalt as $CoCl_2.6H_2O$. was toxic to chicken in 200ppm and can reduced growth. Cooper and cobalt were accumulated in higher concentration in liver , gizzard and feather while muscle was accumulated less concentration of Cu same observation was record by (27) chicken tissues were accumulated higher levels of copper and cobalt in liver , gizzard and feather while less levels found in muscle. Present study revealed that weight of chicken organ (liver, gizzard and heart) were influence by dietary supplemented with copper and cobalt concentration this in agreement with (5)who found high level of copper can effect liver weight and function.

التأثيرات السمية للنحاس والكوبالت المضافة للعلائق على وزن الجسم والاعضاء الداخلية لدجاج اللحم

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الخلاصة

تهدف الدراسة الحالية الى تحديد التأثيرات السمية للعناصر الثقيلة النحاس والكوبالت للعليقة المضاف اليها على الدواجن عند إضافتها بتراكيز معينة. تم توضيح هذه التاثيرات السمية من خلال تأثيرها على وزن الجسم والأعضاء الداخلية لدجاج اللحم ،وتم الكشف ايضا على تراكم هذه المعادن في أنسجة وأعضاء الدجاج بعد المعاملة . تم معاملة الفراخ بعمر يوم واحد على غذاء مضافا اليه تراكيز مختلفة من هذين المعدنين، حيث اعطيت المجموعة الاولى الغذاء مضافا الية النحاس بتراكيز (500، 1500,1000وحدة عالمية) اما المجموعة الثانية فقد اعطيت الغذاء مضافا اليه الكوبالت بتراكيز (100، 500، 1000 وحدة عالمية) بينما غذيت مجموعة السيطره على نفس الغذاء بدون اضافة تراكيز المعادن . درست الثاثيرات بعد اربعة اسابيع من معاملة الفراخ. بينت نتائج الدراسة بان اضافة النحاس والكوبالت ادى الى الى نقص في وزن الفراخ المعاملة معنويا مقارنة بمعاملة السيطرة ،كما اثرت سلبيا على وزن الكبد في الافراخ المعاملة .وقد ادت المعاملة الى زيادة في تركيزات هذين المعدنين في انسجة الفراخ بعد المعاملة بصورة معنوية.

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