**Growth and Immune Response of Broiler Chicks Fed on Oxidized Oil Containing Diets and Supplemented with Different Copper Sources and Levels**

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**Abstract**

Six weeks feeding trial investigated the comparative evaluation of the different copper (Cu) sources (organic or nanoparticles) supplemented at 100 % or 50 % of recommended requirements replacing inorganic form with fresh or oxidized oil on the growth performance, immune response, antioxidant enzymes and hiver histopathology of broiler chickens. Birds were divided into 10 groups including group1(G1) which received the basal diet (BD) contain fresh oil and supplemented with inorganic Cu (15 mg/kg diet ), G2-G3 received Cu as the organic form (15 and 7.5 mg/kg ) and G4-G5 received Cu as Cu nanoparticles (Cu-NPs) with the same level of the organic form respectively, while groups 6-10 have the same design but the diet contain oxidized oil. Inclusion of oxidized oil in diet supplemented with different Cu sources reduced weight, increased Feed intake (FI) and deteriorated FCR when compared with those received fresh oil. Higher level of organic Cu reduced weight, while lower level or both levels of Cu-NPs non-significantly (P> 0.05) increased it. Organic Cu supplementation reduced FI, while nano Cu increased it. 50 % supplementation (7.5mg/kg diet) of requirement as organic Cu significantly (P˂ 0.05) improved average FCR. Supplementation of organic Cu or 50% Cu-NPs improved phagocytosis, lysosomal and bactericidal activity, however higher supplementation level of Cu-NPs reduced them. Inclusion of oxidized oil with different Cu sources had no adverse effect on previously mentioned immune parameters. Organic Cu or Cu-NPs with fresh or oxidized oil non-significantly (P> 0.05) increased serum MDA activity. Organic Cu supplemented birds with fresh or oxidized oil showed reduced GPx and SOD activities, while Cu-NPs increased it. Organic Cu or Cu-NPs decreased *E. Coli* and lactobacilli count in broiler chick's cecum. Organic or nano-Cu supplementation in oxidized oil containing diet reduced both hepatic vacuolar degeneration and inflammation.

**Keywords**: Broiler, Organic, Nanoparticles, copper, Oxidized, oil, Growth, Immune

1. **Introduction**

The use of supplemental fats and oils as dietary energy-yielding ingredients has become a common practice in the poultry feed industry. They are added in the diet to meet the energy requirement of birds especially for those of rapid growth as broilers. However, their inclusion level in the ration is limited because of the high risk of oxidation. Unsaturation degree of fatty acids affects its susceptibility to lipid peroxidation during heat-treatment ([Cleland, James, & Proudman, 2006](#_ENREF_10)). Unsaturated fatty acids are more susceptible to oxidation resulting in the production of peroxide free radicals and lipid peroxidation, as these are undesirable products with offensive odor and toxic properties ([Zhang, Saleh, Chen, & Shen, 2012](#_ENREF_58)). Previous investigations reported the adverse effects of using oxidized oils on the performance of different animals, including broilers. [Dibner, Atwell, Kitchell, Shermer, and Ivey (1996)](#_ENREF_12) reported impaired growth in broilers and swine. Moreover, [Lulin Tan, Rong, Yang, and Zhang (2019)](#_ENREF_52) found that  supplementation of oxidized oils had an adverse effects on the liver and gut barrier function . [Zduńczyk, Jankowski, and Koncicki (2002)](#_ENREF_57) suggested that growth retardation might be due to reduced feed consumption due to reduced palatability and off flavor of rancid feed, and reduced digestibility of the oxidized oil**.** Therefore, the quality of lipid source included in formulated feed is very important, to avoid the oxidative rancidity which could occur and its associated concerns.

Copper (Cu) is a trace element that plays an important role in poultry nutrition. It is necessary for many metabolic processes occur inside the body ([Scott, Vadalasetty Krishna, Chwalibog, & Sawosz, 2018](#_ENREF_49)), an essential component of various proteins and metalloenzymes, critical element for iron metabolism, hemoglobin synthesis, and erythrocyte production. Copper has a fundamental role in antioxidant defense system of body through activation and being a part of antioxidant enzymes ([Djoko, Ong, Walker, & McEwan, 2015](#_ENREF_13)). Cu is mostly provided in the form of inorganic salts which is the widely available source, in higher amounts than the recommended chicken requirement of 15 mg/kg diet (nutrient specification catalogue for commercial breeds). The major part of dietary Cu is not absorbed by birds, but excreted, causing environmental pollution ([Leeson, 2009](#_ENREF_28)). However, there are some variation in the level of absorption between diﬀerent Cu sources. Several studies have compared the diﬀerent Cu forms (organic, inorganic and nano) in terms of their effect on the growth performance, immune response, blood biochemical constituents ([Cheng et al., 2011](#_ENREF_9); [S. El-Kassas et al., 2018](#_ENREF_15); [Jarosz, Marek, Gradzki, Kwiecien, & Kaczmarek, 2018](#_ENREF_22); [A. Scott et al., 2018](#_ENREF_48); [Wang, Cerrate, Coto, Yan, & Waldroup, 2007](#_ENREF_53)). However, literature review dealing with the effect of different Cu sources with oxidized oil in broiler chicken diets and its effect on performance and immune response is very rare. We hypothesized that different Cu sources supplementation could help in amelioration of the adverse effects of dietary inclusion of oxidized oil. So, the main aim of this study was to investigate the effects of dietary replacement of inorganic Cu with the same levels (100 % of the recommended requirements) or lower (50 % of recommended requirements) of organic Cu or Cu-NPs with fresh or oxidized oil source on growth performance, immune response, intestinal microbiota and liver histopathology in broiler chicks.

1. **MATERIAL AND METHODS**
   1. **Birds accommodation and experimental design**

Four hundred and of one-day-old unsexed *Cobb* chicks were used in this trial. They were obtained from Cairo poultry company. Birds were individually weighed (average body weight about 39.6 g), randomly allocated into 10 groups with two replicates / group (20 chicks per each). Chicks of all groups were fed on the experimental basal diet containing similar nutrient content with different copper sources and levels; chicks of group 1 were fed basal diet (BD) containing fresh oil and supplemented with 100 % (15 ppm) inorganic Cu according to breed recommendation of Cu requirements, G2 and G3 were fed the BD contain 100 and 50% of organic Cu (15 and 7.5 ppm) respectively, while chicks of G4 and G5 were fed BD contain 100 and 50% of Nanno Cu (15 and 7.5 ppm) respectively; while groups 6-10 fed the same design of the first 5 groups with the replacement of fresh oil with oxidized oil. Chicks were housed in a clean well-ventilated room and had *ad libtum* feed and water for 6 weeks experimental period. The BD used was formulated to meet the nutrient requirement of poultry ([NRC, 1994](#_ENREF_36)) as presented in table 1 followed by the chemical analysis done according to ([AOAC., 1990](#_ENREF_4)). Copper sources used in this experiment included, copper oxide (CuO) as fed basis produced by El-Gomhoria Co. Egypt (minimum of 80% Cu); the organic form as copper polysaccharide complex (Quali Tech, Chaska, MN) (minimum of 30% Cu) and the copper oxide nano particles produced by Mknano Co. "M K Impex Corp, Canada" with 30nm size particle . On the other hand, oil source useda mixture ofsunflower and cotton seed oil as the fresh form and recovered frying sunflower and cotton seed were obtained from local restaurants as the oxidized form. Chicks were vaccinated against Newcastle disease at day 7, 17, 28 and infectious bursal disease IBD at day 12 and 22 of experiment in the drinking water. Birds body weight (BW) and feed intake (FI) were weighed at weekly intervals and weight gain and feed conversion ratio (FCR) were calculated accordingly. Bird management followed during the experiment was approved from the Animal Care and Ethics Committee at the Faculty of Veterinary Medicine, Matrouh University, Egypt.

* 1. **Sample collection and measurements**

At the end of the feeding trial (6 weeks of age), four birds from / group (two / replicate) were randomly selected. Two blood samples were collected, one was collected one sodium citrate for phagocytosis and the other was without anticoagulant for the separation of the serum. Blood samples were centrifugated at 3000 rpm for 10 minutes. Serum was kept in freezer at -20℃ until used for the analysis of the following biochemical constituents; some antioxidant enzymesas catalase (CAT), superoxide dismutase (SOD), glutathione peroxide (GPx) and lipid peroxidase (Malondialdehyde "MDA"), and serum lipid profile parameters including total cholesterol (TC), triglyceride (TG), low and high density lipoprotein (LDL and HDL) using spectrophotometer using commercial kits produced by Biodiagnostic (Diagnostic and Research reagents). Another serum sample was used for the determination of some immune response parameters as lysosomal activity according to ([Engstad, Robertsen, & Frivold, 1992](#_ENREF_17)) , bactericidal activity ([Rainger & Rowley, 1993](#_ENREF_43)) and phagocytic activity and index according ([Kawahara, Ueda, & Nomura, 1991](#_ENREF_24)) using blood samples with anticoagulant.

After complete bleeding of the birds, liver specimen from each bird was collected for histopathological examination. Samples were washed with distilled water then fixed in 10% formalin for at least 2 days. Slides were prepared and stained with Hematoxylin and Eosin (H and E) for examination ([Bancroft, Layton, & Suvarna, 2013](#_ENREF_6)). After separation of the cecum, one gram of cecal content was removed from the bird. To determine the colony forming units (CFU), one gram of the cecum contents was added to 9 ml peptone water (tube number one), then the solution was well mixed. Ten-fold serial dilution was done according to ([Mountzouris et al., 2007](#_ENREF_34)). Samples were placed on a plate containing MacConkey, and Rogosa medium for growth of *Escherichia coli* and *lactobacillus* bacteria respectively. Cultured samples were incubated at 37°C for 24 h. Observed colonies between 25 to 300, which can be counted was selected as an appropriate dilution, and after being counted it was multiplied by their inverse dilution and the number of bacteria was obtained.

The obtained data were statistically analyzed with analysis of variance ([SAS, 2004](#_ENREF_47)) to assess the significant differences between the different experimental groups for all studied variable. Significance was considered at *P*< 0.05. Results were presented as mean values ± SEM (standard error of the mean).

1. **Results and Discussion** 
   1. **Growth Performance**

Higher level of organic Cu numerically reduced final weight, while the lower level or both levels of Cu-NPs (100 and 50 % of recommended Cu req.) non-significantly (P> 0.05) increased the final BW (table 2). Similarly, [Jegede, Oduguwa, Bamgbose, Fanimo, and Nollet (2011)](#_ENREF_23) observed that birds fed on organic Cu had a significant higher BW compared with those receiving Cu sulphate. Furthermore, [Seham El-Kassas et al. (2019)](#_ENREF_16) documented that CuO-NPs at 50% of the recommended Cu requirement significantly improved growth, as it improved birds BW especially those under heat stress conditions. However, ([Otowski, Ognik, & Kozłowski, 2019](#_ENREF_38)) found that replacement of CuSO4 with Cu-NP as supplemental Cu was decreased from 20 to 10 mg/kg or even 2 mg/kg of diet did not affect the growth of turkeys. The present result suggested that 7.5 mg of organic or nano Cu support higher BW of broiler chicken over the inorganic form which indicate the superiority of these sources over the inorganic when replaced it.

Inclusion of oxidized oil in broiler diet supplemented with inorganic Cu or 7.5 mg of nano Cu non-significantly (P> 0.05) decreased BW, while with organic Cu or 15mg of nano Cu/kg diet reduced (P˂0.05) BW when compared with those received fresh oil. The adverse effects of the thermally oxidized oil may be associated with toxicity of lipid peroxides and reduced biological value of oil ([Bou, Codony, Baucells, & Guardiola, 2005](#_ENREF_7)),highly toxic and readily absorbed lipid peroxidation products ([Kumagai et al., 2004](#_ENREF_27)) which affect the function of the internal organs. Another possible reason could be reduced availability of fat-soluble vitamins in rancid oil which affect the availability of other nutrients, in turn impair growth performance ([Lin et al., 1989](#_ENREF_31)).

Organic Cu supplementation reduced FI, while nano Cu increased it. Inclusion of oxidized oil instead of fresh oil with different copper sources and levels increased total FI compared with those fed on the same diet with fresh oil. The present finding of increased feed consumption with oxidized oil could be attributed to the reduced energy availability required for bird growth due to reduced biological value of this oil source, so the birds attempt to obtain energy required through increased FI. The obtained result are in line with ([L Tan, Rong, Yang, & Zhang, 2018](#_ENREF_51)) replacement of fresh soybean oil by oxidized oil increased FI of broiler chicken while, in contrast with ([Anjum, Mirza, Khan, & Azim, 2004](#_ENREF_3); [Lulin Tan et al., 2019](#_ENREF_52)) as FI was not affected with oxidized oil dietary inclusion.

Moreover, using 100 % (15mg/kg diet) of Cu requirement as organic or nano Cu and 50% (7.5mg/kg diet) of requirement as Cu-NPs had no effect (P> 0.05) on average FCR, however, 50 % (7.5mg/kg diet) of requirement as organic Cu significantly (P˂ 0.05) improved average FCR (table 2). This improvement suggests better utilization of the organic Cu supplemented diets. Similar findings of improved FCR with organic source were reported by ([Jegede et al., 2011](#_ENREF_23); [Lensing & Van der Klis, 2006](#_ENREF_29)). In addition, [Otowski et al. (2019)](#_ENREF_38) observed that replacement of CuSO4 with different levels of nano Cu had no significant effect of FCR in growing turkey. Moreover, inclusion of oxidized oil instead of fresh oil with inorganic Cu or both levels of organic or Cu-NPs significantly (P˂ 0.05) deteriorated average FCR. Birds received with diets contain oxidized oil without or with Cu forms showed lowered body gain with increased FI when compared with those received the same diet with but with fresh oil. These changes in body gain and FI had an adverse effect on the FCR as it was deteriorated as mentioned before. Likewise, ([L Tan et al., 2018](#_ENREF_51)) as FCR was significantly increased with oxidized oils fed birds.

* 1. **Immune response (Phagocytosis, lysosomal and bactericidal activities)**

As shown in table 3, inclusion of oxidized oil instead of fresh oil with different Cu sources and levels had no adverse effect on PA, PI, lysosomal activity and bactericidal activity of broiler chicken compared with those fed the same diet with fresh oil. In addition, Supplementation of organic Cu with both levels or 50% Cu-NPs improved PA, PI, lysosomal activity and bactericidal activity, however higher supplementation level of Cu-NPs reduced the mentioned immune parameters.

Additionally, supplementation of these alternative sources of Cu as organic or nano in broiler ration increased serum copper levels (table 3). Inclusion of oxidized oil instead of fresh oil with different copper sources and levels non significantly (P> 0.05) increased serum Cu concentration, compared with those received fresh oil. These result is consistent with ([S. El-Kassas et al., 2018](#_ENREF_15)) as higher Cu concentration was found with CuO-NPs fed birds than inorganic Cu fed ones. Also, [Ognik, Stepniowska, Cholewinska, and Kozlowski (2016)](#_ENREF_37) reported that increased Cu content in plasma of birds increased with increasing Cu-NPs concentration. As the serum Cu levels in broiler chicken depending on the increased supplementation levels of organic or nano, suggesting that Cu is readily available and absorbable from organic or nano sources than inorganic source.

Copper as an essential trace element, has been reported to have different immune regulating functions such as controlling leukocyte development and function, phagocytic activity, and inducing proinflammatory immune responses ([Goel, Bhanja, Mehra, Majumdar, & Pande, 2013](#_ENREF_18)). Moreover, Cu deficiency has been reported to reduce the PA and PI ([Djoko et al., 2015](#_ENREF_13)). One possible reason for the obtained result of improved immune response with 7.5mg/kg diet (50 % of requirement) with organic or Cu-NPs may be related to elevated serum Cu concentration. The increased serum lysozyme is a good indicator of immune system stimulation as it might contribute to the increased PA, 50% of breed demand supplementation of Cu-polysaccharide complex or CuO-NPs might have a stimulatory effect to the chicken immune response. However, higher level of organic or nano Cu suppress broiler immunity. Consistently, ([S. El-Kassas et al., 2018](#_ENREF_15)) reported that CuO nanoparticles significantly improved broiler immune response through increased levels of PA, lysozyme serum activity as well as upregulating of immune regulating genes. Some previous studies have demonstrated that Cu-NP could induce inflammatory responses, due to their physicochemical properties ([Manolova et al., 2008](#_ENREF_33)). It is expected that Cu ions at high Cu-NP doses lead to an increase in inflammatory response ([Pettibone et al., 2008](#_ENREF_41)). Though, Cu-NP injected into chicken embryos did not show immune stimulatory properties ([Pineda, Sawosz, Vadalasetty, & Chwalibog, 2013](#_ENREF_42)). So that exposure to higher levels of nanoparticles may induce toxic effect, resulting in impaired immune function.

* 1. **Blood serum some oxidative and antioxidant enzyme activities**

Supplementation of both levels from organic Cu or Cu-NPs of broiler ration with fresh or oxidized oil non-significantly (P> 0.05) increased serum MDA activity. Organic Cu supplemented birds with fresh or oxidized oil showed reduced GPx and SOD activities, while Cu-NPs increased it when compared with their reference groups (table 4). Likewise, [Ajuwon et al. (2011)](#_ENREF_1) who found a reduced (*P*< 0.05) activities of SOD or CAT in the erythrocyte and liver of Cu exposed birds. While inconsistent with ([Aksu, Aksu, 횜zsoy, & Baytok, 2010](#_ENREF_2)) who observed reduced plasma MDA activity with organic mineral supplemented groups; [Seham El-Kassas et al. (2019)](#_ENREF_16) enhanced SOD, CAT and GPx enzyme activities with Cu-NPs supplementation during heat stress.

Malondialdehyde (MDA) is an endogenous genotoxic product ([Niedernhofer, Daniels, Rouzer, Greene, & Marnett, 2003](#_ENREF_35)), which considered as an indication of lipid peroxidation and presence of ROS in tissues resulting in its damage ([Liang, Jiang, Mo, Zhou, & Yang, 2015](#_ENREF_30)). Higher concentration of MDA in serum of chicken received oxidized oils could be attributed to mutagenic effects of lipid peroxidation products produced in oxidized fish oil ([Niedernhofer et al., 2003](#_ENREF_35)). Our results indicate that the dietary inclusion of oxidized oil caused oxidative damage influenced bird ability to resist oxidative stress. Moreover, serum SOD and CAT activity in the present study were increased in chicken fed oxidized oil and supplemented with Cu-NP suggesting that Cu from this source help in ameliorating the adverse effects of oxidized oil.

* 1. **Serum lipid profile**

As shown in table 5, different levels of organic Cu or Cu-NPs in broiler ration had no significant effect on serum TG, TC, HDL, LDL and VLDL concentrations. Moreover, all chicks’ groups fed on these Cu sources supplemented diets resulted in non-significantly (P> 0.05) lower CHO/HDL ratio compared with control. The results of the blood lipids analysis are considered to be within the normal range for a healthy chicken. Generally, our results revealed that providing Cu from inorganic, organic or nano sources had no clear effect on blood lipids profile. Unlike the obtained result, ([Abdullah Scott et al. (2018)](#_ENREF_49); [Zahedi, Ghalehkandi, Ebrahimnezhad, & Emami, 2013](#_ENREF_56)) found a reduction in TC levels after adding Cu to poultry diets. The difference may be related to high Cu supplementation in these studies compared with our trial. This could explain that adding higher levels of Cu would regulate cholesterol biosynthesis indirectly by decreasing the reduced form of glutathione and increasing the oxidized form of glutathione ([Bakalli, Pesti, Ragland, & Konjufca, 1995](#_ENREF_5); [S. Kim, Chao, & Allen, 1992](#_ENREF_26))

Inclusion of oxidized oil instead of fresh oil with different copper sources and levels had no effect on serum TG and HDL concentrations however, non-significantly (P> 0.05) increased serum TC and LDL concentrations except higher level of organic Cu (15mg Cu/kg) significantly (P˂ 0.05) increased serum TC and LDL concentrations compared with broiler chicken group fed on the same diet with fresh oil. The present result is supported by ([Özpinar, Örmen, & Firat, 2001](#_ENREF_39)) who found that oxidized oil non significantly increased serum TC concentration and reduced serum TG. The level of blood cholesterol in chickens depend on cholesterol biosynthesis in liver and cholesterol content in diets ([Griminger, 1986](#_ENREF_19)). As previously reported, peroxidation products in oxidized oils may accumulate in liver microsomes and mitochondria of broilers impairing cholesterol metabolism ([Iritani, Fukuda, & Kitamura, 1980](#_ENREF_21)), also could change enzyme activities ([Sallmann, OROMMER, & SOLORO, 1988](#_ENREF_46)). In the present study, cholesterol biosynthesis may have been higher depending on the HMG-CoA reductase activity due to accumulation of peroxidation products.

* 1. **Intestinal Microbiota**

Organic Cu or Cu-NPs significantly (P˂ 0.05) decreased *E. Coli* count and non-significantly (P> 0.05) reduced lactobacilli count in broiler chicks cecal contents. On the other hand, inclusion of oxidized oil instead of fresh oil had no significant effect on E. Coli or lactobacilli counts compared with broiler chicks fed on the same diet with fresh oil except with inorganic Cu source.

A significant decrease in the population of coliform bacteria was found by ([Yoon, Hoon Byeon, Park, & Hwang, 2007](#_ENREF_55)) indicated that dietary addition of inorganic Cu/montmorillonite nanoparticles decreased the numbers of *Escherichia* and *Clostridium perfringens* in the small intestine and cecum. Dietary inclusion of 20 mg/kg Cu as Cu silicate nanoparticles resulted in a significant reduction in coliform bacteria ([Shi, Zheng, Guo, & Zhan, 2013](#_ENREF_50)),the same was the case if 37 mg/kg Cu as Cu-Montmorillonite was added to the diet ([Xia, Hu, & Xu, 2004](#_ENREF_54)).

Generally, the effect of dietary Cu supplementation on the intestinal coliform bacteria seems to be rather weak, the exception may be noticed with organic Cu or Cu nanoparticles that seems to have an effect at concentrations below 50 mg/kg Cu ([Shi et al., 2013](#_ENREF_50)).The present study is in contrast with addition of Cu-propionate corresponding to Cu amount of 100 mg/kg to broiler diets resulted in an increase in the population in study([Paik, Kim, & Park, 2008](#_ENREF_40))while agree with addition of 100 mg Cu-propionate /kg in layer ration resulted a significant reduction in the population of lactobacilli([C. H. Kim et al., 2014](#_ENREF_25)).

Previously, the bactericidal effects of Cu-NP on strains of E. coli and Staphylococcus aureus were demonstrated ([DeAlba-Montero et al., 2017](#_ENREF_11)), as this activity depends on nanoparticles characteristics ([Rakhmetova et al., 2010](#_ENREF_44)), and could be triggered by initiating ROS production after nano-Cu administration ([Chang, Zhang, Xia, Zhang, & Xing, 2012](#_ENREF_8)). One possible explanation for this antibacterial activity could be due to their ability to attach to the bacterial membrane and disturbing its integrity ([Rudramurthy, Swamy, Sinniah, & Ghasemzadeh, 2016](#_ENREF_45)).

* 1. **Liver histopathology**

Broiler chickens fed on inorganic Cu supplemented diet showed normal hepatocytes in acinar-like structures while replacement of inorganic Cu with both levels of organic or nano sources had no effect on liver tissues histopathology except higher level of nano Cu showing mild vacuolation consistent with glycogen storage (figure 1). However, the changes may just be too normal glycogen and lipid accumulation, rather than a degenerative response. On the other hand, liver tissues of broiler chicks fed the BD containing oxidized oil and inorganic Cu showed marked vacuolation of hepatocytes, multifocal areas of mononuclear inflammatory cells infiltration and slight congestion (figure 2). On the other hand, replacement of inorganic Cu by different levels of organic or nano reduced both hepatic vacuolar degeneration and inflammation. Copper Nanoparticles was more effective than organic source to release the toxic effect of oxidized oil on hepatic cells as showing mild degree of hepatic vacuolation mostly towards the normal limits.

The present finding are in line with ([Liu et al., 2009](#_ENREF_32)) as the highest dose of Cu nanoparticles (40 mg/kg BW) caused hepatocyte necrosis. [Doudi and Setorki (2014)](#_ENREF_14) showed liver vasculature in the central veins and portal triad vessels as well as disappearance of hexagonal liver lobules in all groups receiving CuO nanoparticles. The excess of Cu-NP can accumulate in hepatocytes which activate the vacuolation process, which could increase with increasing the amount of CuO-NP leading to limitation of the normal position of the cell nucleus negatively affecting the cell, and even leading to its death ([Gupta et al., 2016](#_ENREF_20)).

**Conclusion**

According to the obtained results, it could be summarized that dietary replacement of inorganic Cu by 50% of recommended requirement from organic or nano source (7.5mg/ kg diet) improve broiler chicken growth performance and immune response. Moreover, inclusion of oxidized oil instead of fresh oil impaired broiler growth, induced oxidative status indicated by increased serum MDA concentration and reduced activities of antioxidant enzymes as well as hepatic vacuolar degeneration and inflammation. Cu- NPs were more effective in reducing the adverse effects on liver cells resulted from feeding on oxidized oils.

**Conflict of interest**

All authors declare that there was no conflict of interest.

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#### **Table 1. Ingredients composition of the used basal diets.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ingredients (%)** | **Starter** | **Grower** | **Finisher** |
| Yellow Corn | 57.45 | 62.6 | 65.2 |
| Soybean meal (44%) | 30.0 | 26.0 | 24.0 |
| Corn gluten (60%) | 7.0 | 6.0 | 5.0 |
| Vegetable oil1 | 1.75 | 2.0 | 2.25 |
| DCP2 | 1.4 | 1.3 | 1.3 |
| Limestone3 | 1.4 | 1.4 | 1.3 |
| Lysine4 | 0.15 | 0.2 | 0.2 |
| DL-Methionine5 | 0.15 | 0.10 | 0.10 |
| Choline Chloride | 0.1 | 0.1 | 0.1 |
| Common Salt | 0.25 | 0.25 | 0.25 |
| Premix (vitamin)6 | 0.15 | 0.15 | 0.15 |
| Mineral premix 7 | 0.1 | 0.1 | 0.1 |
| Anticoccidial | 0.05 | 0.05 | 0 |
| Mycotoxin binder | 0.05 | 0.05 | 0.05 |
| **Chemical composition (%)** |  |  |  |
| Moisture  Crude protein  Ether Extract  Crude fibre  Ash  NFE\*  Calcium  Total phosphorus  Methionine  Lysine  ME Kcal/kg diet  Calorie/protein ratio\*\* | 12.04  22.05  4.88  2.79  6.09  52.15  0.98  0.69  0.57  1.32  2985.3  135.39 | 12.20  20.60  5.55  2.35  5.68  53.62  0.92  0.68  0.46  1.09  3040.1  147.58 | 12.09  18.84  6.05  2.67  5.66  53.26  0.88  0.62  0.44  1.03  3075.2  163.22 |

1Vegetable oil (mixture of sunflower oil and cottonseed oil), 2DCP= dicalcium phosphate (contain 18% P and 25% Ca), 3Limestone (contain 34% calcium),4Lysine = lysine hydrochloride (contain 98.5% Lysine), 5DL-Methionine (Produced by Evonic Co and contain 99.5% methionine).6The premix used was Heromix produced by Heropharm and composed of (per 1.5 kg) vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, thiamin 1000 mg, riboflavin 5000 mg, pyridoxine 1500 mg, cyanocobalamin 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid.7Mineral premix was formulated and composed of (1 kg): 100000 mg Manganese, 100000mg Zinc,15000mg Copper, 40000 mg Iron, 1000 mg Iodine, 350 mg Selenium and 150 mg Cobalt and calcium carbonate as carrier up to 1 kg.

\* NFE= Nitrogen free extract (calculated by difference "100- (moisture % + CP % + EE % + CF % + ash %)".

\*\*\*Calorie/protein ratio = ME Kcal/CP%

**Table 2. Growth performance of broiler chicken affected by different sources and levels of copper with fresh or oxidized oil.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | copper sources and levels | Oil types | |
| Fresh oil | Oxidized oil |
| Initial body weight (g/chicks) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 39.61±0.34ax  39.61±0.30ax  39.59±0.29ax  39.81±0.30ax  39.95±0.29ax | 39.68±0.34ax  39.46±0.33ax  39.49±0.33ax  39.62±0.31ax  39.56±0.30ax |
| Final body weight (g/chicks) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 1961.81±47.38ax  1924.47±42.74ax  2060.26±55.78ax  1965.16±53.88ax  2034.33±43.91ax | 1812.63±66.24abx  1760.59±55.61by  1834.56±50.43aby  1745.00±33.38by  1936.11±56.30ax |
| Total body gain (g/chicks) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 1916.19±46.09ax  1885.67±42.50ax  2021.32±55.58ax  1925.97±53.62ax  1994.72±43.64ax | 1774.17±65.90abx  1722.59±55.38bx  1796.15±50.15aby  1706.13±33.13by  1897.57±56.05ax |
| Total feed intake (g/chick) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 3844.78  3704.76  3704.66  3900.22  4029.36 | 4432.34  4338.49  4343.51  4368.71  4437.57 |
| Average feed conversion ratio (FCR) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 2.04±0.05ay  2.00±0.05aby  1.87±0.05by  2.10±0.06ay  2.06±0.05ay | 2.59±0.09ax  2.56±0.07ax  2.47±0.07ax  2.59±0.05ax  2.40±0.08ax |

Values are means ± standard error. Mean values with different letters at the same column (a - d letters) or row (x – z letters) differ significantly at (*P*˂ 0.05).

**Table 3. Phagocytosis, lysosomal and bactericidal activities of broiler chicken as affected by different sources and levels of copper with fresh or oxidized oil.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | copper sources and levels | Oil types | |
| Fresh oil | Oxidized oil |
| Phagocytic activity | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 36.73±1.20cy  41.15±0.65bx  45.03±0.66ax  32.53±0.84dy  44.93±0.84ax | 40.28±0.69bx  42.03±0.84bcx  48.18±1.00ax  37.65±1.47cx  45.78±0.95ax |
| Phagocytic index | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 1.80±0.12bx  2.07±0.06bx  2.27±01.09ax  1.32±0.12cy  2.22±0.14ax | 2.05±0.06bx  2.10±0.08bx  2.44±0.08ax  1.85±0.10bx  2.23±0.09ax |
| Lysosomal activity | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 0.24±0.03ax  0.28±0.08ax  0.30±0.03ax  0.22±0.05ax  0.27±0.04ax | 0.25±0.03ax  0.34±0.06ax  1.92±1.70ax  0.25±0.03ax  0.14±0.02ax |
| Bactericidal activity | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 39.58±1.50ax  38.90±2.34ax  35.48±0.91ax  39.20±1.50ax  37.13±1.09ax | 39.15±1.39bx  37.45±1.56bx  42.73±2.30abx  40.38±0.98abx  43.48±0.63ax |
| Copper (mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 127.00±3.14dx  147.50±2.60abx  133.75±2.06cdx  156.25±3.20abx  142.25±4.07bcx | 125.25±2.39dx  147.50±4.09bcx  148.50±5.25bcx  163.00±4.53ax  150.00±4.74bcx |

Values are means ± standard error. Mean values with different letters at the same column (a - d letters) or row (x – z letters) differ significantly at (*P*˂ 0.05).

**Table 4. Blood serum some oxidative and antioxidant enzyme activities of broiler chicken affected by different sources and levels of copper with fresh or oxidized oil.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | copper sources and levels | Oil types | |
| Fresh oil | Oxidized oil |
| MDA  (nmol / ml) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 10.11±0.43  10.52±0.30  10.86±0.30  10.69±0.23  10.78±0.29 | 10.84±0.40  10.82±0.24  11.03±0.21  10.70±0.35  10.77±0.13 |
| GPx  (u/ml) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 33.14±1.84abx  29.69±1.04bx  28.86±1.78bx  36.67±1.34ax  33.06±0.92abx | 29.70±2.42abx  26.66±1.59bx  24.74±1.07cx  32.85±0.91ax  32.23±0.67ax |
| SOD (u/ml) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 228.13±53.13  187.50±25.52  156.25±54.13  250.00±25.52  221.25±18.04 | 211.25±40.34  203.13±29.92  234.38±29.92  250.00±36.08  265.63±46.88 |
| CAT (u/L) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 83.01±8.84  53.93±23.42  58.74±19.88  123.87±55.71  63.54±26.25 | 30.81±12.12  34.66±11.87  31.90±12.03  37.55±8.60  31.31±10.29 |

Values are means ± standard error. Mean values with different letters at the same column (a - d letters) or row (x – z letters) differ significantly at (*P*˂ 0.05).

Table 5. Blood serum lipid profile of broiler chicken as affected by different sources and levels of copper with fresh or oxidized oil.

|  |  |  |  |
| --- | --- | --- | --- |
|  | copper sources and levels | Oil types | |
| Fresh oil | Oxidized oil |
| Triglycerides (mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 207.67±1.28  206.51±1.02  205.73±1.76  208.12±0.58  207.48±1.32 | 206.70±1.39  207.48±0.61  205.99±1.06  205.67±1.43  206.19±1.19 |
| Total cholesterol (CHO mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 208.15±7.67ax  205.24±10.32ay  222.09±6.24ax  216.23±4.01ax  217.84±9.18ax | 220.41±6.23ax  230.32±0.30ax  221.17±4.83ax  224.60±1.93ax  227.09±1.50ax |
| HDL (mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 50.88±1.15  53.40±1.51  55.12±1.87  52.83±1.99  55.50±1.97 | 51.21±2.70  54.13±0.94  50.30±0.27  52.41±0.67  52.65±1.45 |
| LDL (mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 115.74±6.84ax  110.54±9.72ay  125.83±5.62ax  121.78±4.75ax  120.84±8.38ax | 127.86±8.04ax  134.70±0.66ax  129.67±4.68ax  131.05±1.36ax  133.21±2.73ax |
| VLDL (mg/dl) | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 41.53±0.26  41.30±0.20  41.15±0.35  41.62±0.12  41.50±0.26 | 41.34±0.28  41.50±0.12  41.20±0.21  41.13±0.29  41.24±0.24 |
| CHO/HDL ratio | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 4.09±0.12  3.85±0.18  4.05±0.15  4.11±0.18  3.93±0.14 | 4.35±0.31  4.26±0.07  4.40±0.07  4.29±0.03  4.33±0.14 |

Values are means ± standard error. Mean values with different letters at the same column (a - d letters) or row (x – z letters) differ significantly at (*P*˂ 0.05).

**Table 6. E-coli and Lactobacilli counts of broiler chicken cecum as affected by different sources and levels of copper with fresh or oxidized oil.**

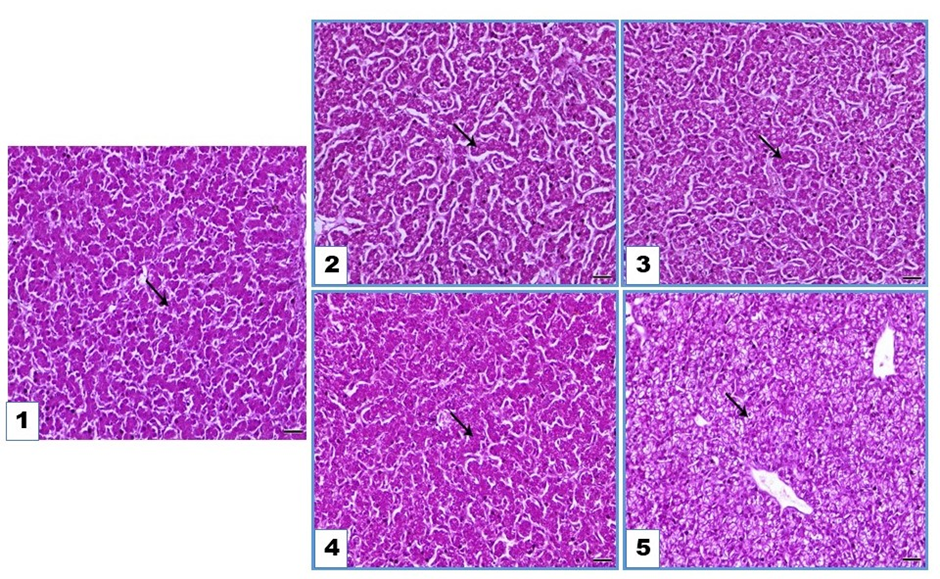
|  |  |  |  |
| --- | --- | --- | --- |
|  | copper sources and levels | Oil types | |
| Fresh oil | Oxidized oil |
| E-coli | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 80.00±27.08ax  7.25±2.43bx  5.25±4.92bx  8.75±5.15bx  12.25±6.26bx | 3.50±0.96ay  23.25±12.74ax  40.25±24.36ax  11.25±0.95ax  8.25±4.80ax |
| Lactobacilli | 15mg/kg (inorganic)  15mg/kg (organic)  7.5mg/kg (organic)  15mg/kg (nano)  7.5mg/kg (nano) | 85.00±18.48  40.00±9.79  57.50±14.93  47.50±18.87  70.00±27.39 | 103.00±41.46  132.50±21.36  75.00±34.03  68.00±44.50  50.00±17.80 |

Values are means ± standard error. Mean values with different letters at the same column (a - d letters) or row (x – z letters) differ significantly at (*P*˂ 0.05).

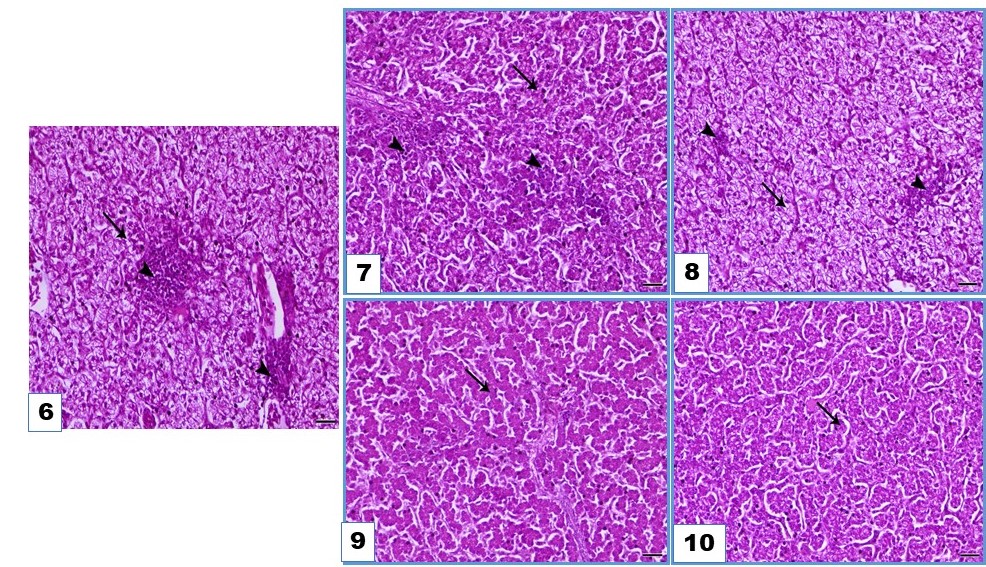
**Figure legend**

**Fig.1** Liver morphology of broiler chicken.**1)** represents broiler chickens fed on 15 mg inorganic Cu/kg and fresh oil showing normal hepatocytes in acinar-like structures (arrow), **2-3)** represents chickens fed on fresh oil containing diet with 15mg (left) or 7.5 mg (right) of organic Cu/kg, showing normal hepatic tissues (arrow) of both groups, **4-5)** represents chickens fed on fresh oil containing diet with 15mg (left) or 7.5 mg (right) of nano Cu/kg, showing normal hepatocytes (arrow indicates mild vacuolation consistent with glycogen storage) and normal hepatocytes arranged in acinar-like manner (arrow) respectively

**Fig.2** Liver morphology of broiler chicken, **6)** represents broiler chickens fed on 15 mg inorganic Cu/kg and oxidized oil) showing marked vacuolation of hepatocytes (arrow) and multifocal areas of mononuclear inflammatory cells infiltration (arrowheads); **7-8)** represents broiler chickens fed on oxidized oil containing diet with 15mg (left) or 7.5 mg (right) of organic Cu/kg, showing decrease both hepatic vacuolar degeneration (arrow) and inflammation (arrowheads indicates mild periportal mononuclear inflammatory cells infiltration) and showing decrease hepatic vacuolation of moderate degree (arrow) and portal inflammation, respectively; **9-10)** represents broiler chickens fed on oxidized oil containing diet with 15mg (left) or 7.5 mg (right) of nano Cu/kg, showing normal hepatocytes in acinar-like structures and showing mild degree of hepatic vacuolation mostly towards the normal limits (arrow) respectively

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**Fig. 1**



**Fig. 2**