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The Effects of Replacing Inorganic with a Lower Level of Organically Complexed Minerals (Cu, Zn and Mn) in Broiler Diets on Lipid Peroxidation and Antioxidant Defense Systems

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ABSTRACT : In this study, the effects of replacing inorganic copper, zinc and manganese with different levels of organic complexes of the same trace minerals on the lipid peroxidation and antioxidant defense systems in broilers were investigated. Two-hundred Ross-308 one-day-old broiler chickens were placed on controlled diets until 42 d of age. The experimental animals were divided into four groups comprising three experimental groups and one control group, each consisting of 50 chickens. All groups were also divided into five subgroups each containing 10 broiler chicks. The mineral content of the control group diet was controlled using a standard inorganic mineral premix with supplement levels and sources of trace minerals typical of commercial broiler diets according to the National Research Council (NRC) (containing 8 mg Cu as $CuSO_4$, 40 mg Zn as $ZnSO_4$, and 60 mg Mn as MnO, per kg). In the experimental diets, mineral premix was also comprised of inorganic formulations, except for those of Cu, Zn and Mn. Organically-complexed Cu, Zn, and Mn were separately added to the basal diet at 1/3 (L1), 2/3 (L2) and 3/3 (L3) levels with respect to the NRC recommendation, as Bioplex Cu^{TM}, Bioplex Zn^{TM}, Bioplex Mn^{TM}. At the end of the trial, the plasma Zn level significantly increased when the plasma Cu level significantly decreased ($p<0.05$) in chickens fed at 2/3 and 3/3 levels of organically complexed minerals. The liver trace mineral concentrations were significantly higher in chickens fed inorganic trace minerals in comparison to those fed organically-complexed minerals. The plasma malondialdehyde (MDA) level of experimental chickens was decreased in groups receiving levels of organic Cu, Zn and Mn in comparison to those fed inorganic forms ($p<0.01$). The erythrocyte superoxide dismutase (SOD) activity was higher in all groups receiving the organic mineral supplements in comparison to those fed inorganic forms ($p<0.01$). No differences were observed on either the erythrocyte catalase (CAT) activity or the plasma ceruloplasmin (Cp) levels, and the liver MDA levels and liver CAT and SOD activities in any of the groups that received the organic supplements of Cu, Zn, and Mn. It was concluded that supplementation of lower levels of organically-complexed copper, zinc, and manganese instead of their inorganic forms in diets had no negative effects on the antioxidant defense system in broilers. (**Key Words :** Organically Complexed Mineral, Lipid Peroxidation, Antioxidant Defense System, Broiler)

INTRODUCTION

In commercial poultry diets, the majority of these trace minerals are supplemented into animal diets as inorganic forms (sulphate or oxide salts). But, inorganic trace minerals can suffer from high rates of loss due to dietary antagonisms. In this context, inorganic trace minerals are also used to supply between two and ten times more than the amounts of recommended by National Research Council (NRC) for animal diets (Inal et al., 2001). However, an excess of supplemental inorganic minerals leads to waste and environmental contamination from excessive excretion (Leeson, 2003).

Use of organically complexed trace minerals can help prevent these losses, due to increased stability in the upper gastrointestinal tract of the animal. Indeed, a variety of trials have demonstrated greater bioavailability of organically complexed trace minerals, which in turn would allow for lower inclusion rates and reduced excretion (Bao et al., 2009). Previous studies (Aydemir et al., 2000; Bulbul et al., 2008) have examined the effectiveness of minerals on lipid peroxidation and the antioxidant system; however,

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they only examined the systemic effects of these minerals individually. In recent years, there has been a considerable reduction in the recommended levels of organically complexed minerals in broiler diets (Bao et al., 2007; Nollet et al., 2007). Yet, there has been little research into the effects of these organically complexed minerals in combination at these lower levels on lipid peroxidation and antioxidant defense system.

Reactive oxygen species (ROS) are essential for proper cell functioning and are widely produced during normal cell metabolism. Low levels of ROS are necessary for many cell-signaling procedures. Under normal physiological conditions, a balance exists between the levels of ROS produced during cellular metabolism and the levels of endogenous antioxidants, which serve to protect tissues from oxidative damage. Imbalance or loss of cellular redox homeostasis results in oxidative stress, causing severe damage to cellular components (Sies, 1991). An excessive level of ROS leads to a variety of pathological conditions, including lipid peroxidation, apoptosis, and tissue damage. Lipid peroxidation can compromise the integrity of cell membranes and increase cell membrane fluidity which adversely affects immune responses**,** and lipid peroxidation inactivate membrane bound receptors and enzymes (Bendich, 1993).

Research has shown maintenance of the redox balance to be important to the health of broiler chicks, due to their high body lipid content, which is the cause of lipid peroxidation (Zhang et al., 2008). To protect against lipid peroxidation and oxidative damage, all living organisms have evolved an interdependent antioxidant system that includes enzymatic and non-enzymatic components in the liver (Ohtsuka et al., 1998) and erythrocytes (Orzechowski et al., 2000). The major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Reduced glutathione (GSH), melatonine, ceruloplasmin (Cp), and albumin are non-enzymatic antioxidants **(**Halliwell and Gutteridge, 1986). Copper, zinc, and manganese are essential trace minerals that are important co-factors of SOD and CAT, and participate in the structure of ceruloplasmin (Haddad et al., 2008). In recent years, nutritionists have been recommended the lower inclusion levels of organically complexed minerals compared to organic forms in animal diets. On the other hand, it is possible that the inadequate mineral intake may increase the susceptibility of tissues to oxidative stress, by causing to a weakness in protective antioxidant systems. For this reason, the present study was investigated to determine whether the replacing inorganic with at lower level of combined organically complexed minerals (Cu, Zn and Mn) affects the lipid peroxidation and antioxidant defense systems in broilers.

MATERIAL AND METHODS

Animals, diets, and experimental design

The experiment was in accordance with Animal welfare, and was conducted under protocols by the Veterinary Faculty in Hatay-Turkey. A total of two-hundred, one-day-old, broiler chickens (Ross-308) were fed controlled diets until they were 42 d of age. The experimental animals were divided into four groups, comprising three experimental groups and one control group, each consisting of 50 broilers. All groups were further sub-divided into five replicates containing 10 broiler chicks each. The birds were given *ad libitum* access to feed and water. A lighting schedule of 23 Light:1 Dark (23L:1D) was imposed throughout the experimental period. Ambient temperature was gradually decreased from 32°C on d 1 to 22°C by the end of the experiment. Newcastle disease vaccination was performed on d 10, and Gumboro vaccination on d 18. The basal diet (Table 1) was formulated according to NRC (1994) recommendations and analyzed by the AOAC (2000). The experiment was divided into two phases:phase one (0-21 d) and phase two (21-42 d).

The mineral content of the control group diet was

Table 1. Composition of the basal diets (%)

	Starter (0 to 21 d)	Finisher (21 to 42 d)
Ingredients (%)		
Maize	51.5	55.2
Wheat	7	7
Wheat bran	4.5	4.5
Extracted soybean meal	27.5	24
Fish meal	5.5	4.3
Vegetable oil	1.5	2.5
Limestone	1	1
Di calcium phosphate	0.75	0.75
NaCl	0.25	0.25
Vit-Min. premix*	0.5	0.5
Calculated nutrients		
ME (MJ/kg)	12.6	13
Crude protein (%)	22.1	20
Ca (%)	0.9	0.8
P (%)	0.6	0.7
Lysine (%)	1.1	0.8
Analyzed nutrients	1.1	0.8
Cu (mg/kg)	9.63	8.71
Zn (mg/kg)	35.65	30.23
Mn (mg/kg)	38.84	36.63

* Supplied per kilogram of diet: Vitamin A 15.000 IU; cholecalciferol 1.500 ICU; vitamin E, 30 IU; menadion, 5.0 mg; thiamin, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg, pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 μg; Mn, 60.0 mg; Zn, 40 mg; Fe, 30.0 mg; Cu, 8.0 mg; I, 2.0 mg; Se, 0.15 mg.

controlled using standard inorganic mineral premix (containing 8 mg Cu as $CuSO_4$, 40 mg Zn as $ZnSO_4$, and 60 mg Mn as MnO, per kg). This was in line with the normal supplementary levels and sources of trace minerals in commercial broiler feeds according to NRC. For the experimental diets, mineral premix was prepared using inorganic forms of all components except for Cu, Zn, and Mn. Organically complexed Cu, Zn, and Mn were separately added to the basal diet at 1/3 (L1), 2/3 (L2) and 3/3 (L3) proportions of their inorganic form, as Bioplex™ (Table 2). Bioplex is an aminoacid-hydrate complex, bonded with Cu, Zn and Mn. Amino acid produced from hydrolized-soy protein. Bioplex Cu™, Bioplex Zn™ and Bioplex Mn™ contain 150.000 mg/kg of Cu, 1.000.000 mg/kg of Zn, and 150.000 mg/kg of Mn, respectively.

The organically complexed Cu, Zn, and Mn were provided as Bioplex-Cu™, Bioplex-Zn™ and Bioplex-Mn™ (Alltech Biotech. Ltd., Dandenong South, Victoria, Australia).

Sample collection and measurements

At the end of the trial, we collected blood samples by venipuncture from the ulnar veins of two broilers randomly chosen from each replicate. We then centrifuged the samples at 3,000 rpm for 15 min to separate the plasma and erythrocytes. We harvested plasma by aspiration. Prior to analysis, we washed the erythrocytes three times with physiological saline and stored them at -20°C until analysis. We used the plasma specimens for the determination of MDA and Cp, and the erythrocyte samples for the determination of SOD and CAT. On the day that blood was collected, we sacrificed two broilers randomly chosen from each replicate by decapitation. The livers were excised and washed in cold ice saline (0.9%). Liver homogenate was made in an ice cold homogenization buffer (0.32 M sucrose, 1 mM EDTA, 10 mM Tris HCl, pH 7.4), and cytosolic samples of liver homogenate were obtained by centrifuging at 10.000 g for 10 min at +4°C.

Plasma and liver malondialdehyde (MDA) levels were determined using the method described by Yoshoiko et al. (1979) based on thiobarbituric acid (TBA) reactivity. We measured optical densities at 535 nm by spectrophotometer (Schimadzu UV 1208). We established the erythrocyte and liver CAT activity using the method of Aebi (1984) with a spectrophotometer at 240 nm. Erythrocyte and liver SOD activities were determined with the method of Sun et al. (1988). This involves inhibition of nitroblue tetrazolium (NBT) reduction by superoxide anions. The resulting reduction of NBT was measured at 560 nm by spectrophotometer. We determined liver protein levels by Bradford reagent using a spectrophotometer at 595 nm. Plasma ceruloplasmin level was determined using PPD (P-Phenilen diamin dichloride). The optical density was measured at 546 nm by spectrophotometer (Colombo and Richterich, 1964). For measurement of trace minerals in plasma, 4 ml of plasma sample was wet-ashed in a tube by adding 10 ml of nitric acid and heated to minimal volume (the solution was never allowed to dry). After the solution was cooled, it was filtered into 25 ml flask and diluted to 25 ml with deionized water. Liver samples were then ashed (550°C for 4 h). Approximately 1 g of liver samples was then dissolved in 10 ml of HCIO4 and boiled for 10 min; the remainder was then mixed with 5 ml HNO_3, then filtered and diluted to 50 ml. with deionized water. All samples were then analyzed using Inductively Coupled Plasma Emission Spectroscopy (ICP); analysis was performed by a laboratory specialising in this assay.

Statistical methods

Data were analyzed with a one-way ANOVA, using the General Linear Models (GLM) procedure of the SAS (SAS, 1994). We used Duncan's Multiple Range Test option of the SAS to separate significant differences between means. All results were based on a significance level of $p<0.05$.

RESULTS

No differences in response to addition of lower levels organic Zn, Cu and Mn instead of their inorganic forms were observed among any of the performance parameters measured.

At the 42 d of age, the plasma and liver trace mineral concentration of chickens fed different diets are given in Table 3. The plasma Mn and Fe levels were not different

Table 2. Source and amounts of trace minerals fed to broilers

Diet	Added Zn (mg/kg)	Added Cu (mg/kg)	Added Mn (mg/kg)
Inorganic (control)*	40	8	60
1/3 organic (L-1)**	13	2,5	20
2/3 organic (L-2)**	26	5	40
3/3 organic (L-3)**	40	8	60

* Supplied per kilogram of diet: Vitamin A 15.000 IU; cholecalciferol 1.500 ICU; vitamin E, 30 IU; menadion, 5.0 mg; thiamin, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 μg; Mn, 60.0 mg; Zn, 40 mg; Fe, 30.0 mg; Cu, 8.0 mg; I, 2.0 mg; Se, 0.15 mg.

** Organically complexed Mn, Zn and Cu were provided as Bioplex-Mn™, Bioplex-Zn™ and Bioplex-Cu™.

Table 3. Plasma and liver trace mineral concentration of chickens fed different diets

Plasma	Diets [1]				SEM	p
	Control	L1	L2	L3		
Zn (μg/ml)	2.87[c]	2.98[bc]	3.16[ab]	3.24[a]	0.05	*
Cu (μg/ml)	0.37[a]	0.39[a]	0.30[b]	0.26[b]	0.01	*
Mn (μg/dl)	0.20	0.28	0.17	0.15	0.30	NS
Fe (μg/ml)	1.04	1.13	0.82	1.01	0.67	NS
Liver						
Zn (μg/ml)	66.18[a]	23.65[c]	24.38[c]	35.77[b]	0.67	**
Cu (μg/ml)	8.79[a]	4.06[bc]	6.1[b]	5.79[b]	0.30	**
Mn (μg/dl)	8.72[a]	5.20[d]	6.56[c]	7.38[b]	0.05	**
Fe (μg/ml)	149.02[a]	76.66[c]	74.81[c]	85.50[b]	0.01	**

Means represent from 10 chickens per treatment

[a, b, c] Means values within a row having differing superscripts are significantly different by least significant differences test ($p<0.05$). NS: non-significant, * $p<0.05$, ** $p<0.01$.

[1] Control: Inorganic Cu, Zn and Mn at NRC recommendation levels as sulfate. L1, L2, and L3: Organically complexed Cu, Zn and Mn at 1/3, 2/3 and 3/3 proportions instead of NRC recommendations levels as Bioplex™, respectively.

between experimental groups. The plasma Zn level significantly increased as the plasma Cu level significantly decreased ($p<0.05$) in chickens fed at 2/3 and 3/3 levels of organically complexed minerals. The liver trace mineral concentrations were significantly higher in chickens fed inorganic trace minerals in comparison to those fed organically complexed minerals ($p<0.01$). On the other hand, the liver trace mineral concentration of chickens fed at 3/3 levels of organically complexed minerals were significantly higher than those others fed at 1/3 and 2/3 levels of organic forms ($p<0.01$). It was important finding that the cumulative liver mineral concentration was also higher in chickens fed inorganic trace minerals in comparison to the other fed same level organically complexed minerals.

The plasma MDA and Cp levels, and the erythrocyte CAT and SOD activities of chickens fed experimental diets are shown in Table 4. All supplemental levels of organically complexed Zn, Cu, and Mn led to decreased plasma MDA levels of experimental chickens in comparison to those fed inorganic forms (control) ($p<0.01$). Plasma MDA levels did not differ between the organically complexed minerals supplemented groups ($p>0.05$). On the other hand, erythrocyte SOD activity increased in all organically complexed minerals supplemented groups in comparison to those fed inorganic forms (control) ($p<0.01$). None of the experimental groups showed any effects from the supplementation of organic Zn, Cu, and Mn in either erythrocyte CAT activity or plasma Cp levels.

Table 4 shows the liver MDA levels and the liver CAT and SOD activity of chickens fed experimental diets. We observed no differences among the treatments in liver MDA levels or liver CAT and SOD activity.

DISCUSSION

Trace elements such as Cu, Zn, Mn and Fe are essential components of the antioxidants superoxide dismutase,

Table 4. The plasma, erythrocyte and liver MDA, Cp, CAT and SOD level of chickens fed experimental diets

	Diet[1]				SEM	p
	Control	L1	L2	L3		
Plasma MDA (μmol/L)	14.17[a]	11.93[b]	12.50[b]	11.93[b]	0.260	**
Plasma Cp (mg/dl)	9.78	9.67	10.02	10.04	0.160	NS
Erythrocyte CAT (K/gHb)	0.53	0.60	0.75	0.45	0.057	NS
Erythrocyte SOD (U/gHb)	1.83[b]	2.60[a]	2.79[a]	3.06[a]	0.129	**
Liver MDA (μmol/L)	18.09	18.70	19.16	19.96	0.318	NS
Liver CAT (U/mg protein)	0.73	0.84	0.82	0.94	0.046	NS
Liver SOD (U/mg protein)	0.94	0.98	0.99	1.03	0.015	NS

Means represent from 10 chickens per treatment

[a, b] Means values within a row having differing superscripts are significantly different by least significant differences test ($p<0.05$). NS: non-significant, ** $p<0.001$.

[1] Control: Inorganic Cu, Zn and Mn at NRC recommendation levels as sulfate. L1, L2, and L3: Organically complexed Cu, Zn and Mn at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals recommend by NRC, respectively.

catalase or ceruloplasmin. Therefore, reducing mineral concentration in diet may also cause to inadequate minerals intake which may increase the susceptibility of tissues to oxidative stress, by causing to a weakness in protective antioxidant systems. In the present study, whether the using at a much lower levels of organically complexed form of copper, zinc and manganese instead of their inorganic forms causes to weakness on the lipid peroxidation and antioxidant defense systems in broiler were investigated.

As the plasma mineral levels of chickens were examined, depending on the increased inclusion levels of organically complexed minerals (L2 and L3), the plasma copper level decreased while the plasma zinc level increased ($p<0.05$). This finding possibility due to interaction between zinc and copper, as previous studies reported that the copper absorption may be prevented by the increasing zinc absorption from intestines (Rowin and Lewis, 2005). Moreover, not only copper and zinc but also mangane and iron are at a race in binding to protein molecules. One of them affects the other's absorption from the intestinal mucosa (Chua et al., 1996). Organically complexed minerals can be more easily absorbed from intestines compared to the inorganic forms, because of their protein/amino acid-related structures. Zinc received with diet increases the production of metallothioneins, which are the metal binding proteins with low-moleculer- weight. On the other hand, copper has an higher affinity to engage the metallothioneins compare to others ion-minerals. More amount of copper is therefore engaged to the metallothioneins and then accumulated in the enterocyte. But, an important part of copper is excreted by shedding of enterocyte into the gastointestinal canal. Thereby, the plasma zinc level increases when the plasma copper level decreases (Harris, 1997).

In the current study, an important finding was observed between the liver mineral concentration of chickens fed completely inorganic minerals and the chikens fed same level organically complexed minerals (Table 3). Despite these two groups received same levels of minerals, the liver mineral concentrations were significantly lower in chickens fed at 3/3 level of organically complexed minerals ($p<0.01$). This may be an important finding for reducing toxication originating from toxic mineral accumulation in the liver such as copper that difficulty excrete from organism.

The balance between the activity and intracellular content of antioxidant enzymes is important for the survival and health of living organisms. When ROS formation is more rapid than the detoxification capacity of cellular antioxidant mechanisms, oxidative stress occurs, resulting in cellular damage **(**Reiter et al., 1997). Enhancement of ROS formation alters the antioxidant enzyme activity of blood and tissue, causing lipid peroxidation. Lipid peroxidation is a chain reaction, initiated by free radical attacks on phospholipids or the polyunsaturated fatty acid of the membranes of cellular and subcellular organelle. This leads to the generation of complex mixture of aldehydes, ketons, and polymerization products that react and destroy the biomolecules, enzymes, and nucleic acids. Malondialdehyde is one of the major secondary oxidation products of peroxidized polyunsaturated fatty acids and the most abundant and efficient markers of lipid peroxidation among reactive aldehydes (Das, 2002).

As the antioxidant status of chickens were assesed, it was found all supplemental levels of these organically complexed minerals decreased lipid peroxidation in the chickens by lowering the plasma levels of MDA (Table 4). The decrease in plasma MDA level could be linked to the enhancing capacity of scavenging free radicals and decreases the damage of tissues or cells by organically complexed minerals. The increase plasma zinc levels and erythrocyte SOD activity support this view (Tables 3 and 4). This observation is important for the using of at a much lower levels of organically complexed minerals and supports the idea that zinc and copper are required the activity of superoxide dismutase. It well knows that, superoxide dismutase is the major antioxidant enzyme containing copper, zinc, and manganese. The copper is involved in catalysis while the zinc is involved in the stability of the enzyme (Forman, 1973). In the current study, the increasing supplementation level of organically complexed minerals (L2 and L3) decreased the plasma copper level (Table 3) but the erythrocyte SOD activities did not decreased despite to this decreasing (Table 4). This could be due to the increased plasma zinc level. It is mentioned that zinc stabiles the red cell membrane against cellular change, cause to peroxidation. The results of the current study are in accordance with the results of the literature mentioned above.

The protective effects of cellular Cu/Zn-SOD against oxidative tissue damage can mainly be attributed to their breakdown of the superoxide radicals into hydrogen peroxide and oxygen, and their decrease of hydroxyl radical formation which is the prime initiators of lipid peroxidation **(**Balevska et al., 1981). Previous studies of dietary supplementation of individual organically complexed minerals have found that a zinc and copper combination is involved in the formation of Cu/Zn-SOD and the level of this enzyme is elevated when these minerals added to the diet **(**Jia-Peng et al., 2001; Şahin et al., 2005). As a matter of fact that a reduction in SOD activity may increase MDA level which predict to oxidative damage in cell membranes and other cellular structures (Balevska et al., 1981). The results of the current study are in accordance with the results of the literature mentioned above.

Aydemir et al. (2000) observed a positive and highly significant correlation in chickens between plasma copper

levels and erythrocyte SOD activity. They found that erythrocyte SOD activity increased in chickens fed with a Cu and Se premix supplement in comparison to those fed no supplemental premix. In the present study, the erythrocyte SOD activity increased in all groups receiving organically complexed mineral supplements in comparison to those fed inorganic forms (control) ($p<0.01$), while the liver SOD activities did not differ among the groups (Table 4). In the current study, although the liver mineral concentrations of the groups received organically complexed minerals were significantly lower in comparison to the control (Table 3), there was no any evidence caused to oxidative damage in the liver cell membrane and other tissues. No changes were observed between groups for the liver MDA level and, the liver SOD and CAT activities (Table 4). This finding stated that the using that low levels of organically minerals (Cu, Zn and Mn) might be sufficient for a healthy liver metabolism.

As far as we are aware, there has been no study published on the effects of organic copper supplementation on plasma ceruloplasmin level in broilers. An important observation in the present study was the lack of significant differences between ceruloplasmin level in the chickens fed at 1/3 or 2/3 levels of organically complexed minerals and the chickens fed organic or inorganic minerals at the levels of NRC (Table 3). Ceruloplasmin is a copper-glycoprotein that shows peroxidase activity. Most changes observed in plasma copper levels are associated with changes in ceruloplasmin, which contains >70-80% of plasma copper (Milne, 1994). This finding supports the hypothesis that organically complexed minerals have more bioavailability than their inorganic forms (Close, 1998). Catalase is a heme-containing antioxidant enzyme, which acts sequentially to SOD in the conversion of hydrogen peroxide to water (Cohen et al., 1970). Fe^{+3} protoporphyrin is the central catalase group. Catalase activity is reduced in Cu deficiency (Lai et al., 1995). This is because Cu is necessary to adequate Fe utilization, which is an important component of catalase. Some researchers have reported an increase (Bozkaya et al., 2001); others have reported a decrease in erythrocyte and liver catalase activity to be associated with copper deficiency (Chevar et al., 1992). In the present study, there was no significant reduction in erythrocyte and liver catalase activity in any of the groups; however, chickens in the L1 and L2 groups were fed a much lower level of organically complexed minerals than those in the control and L3 groups (Table 4). This might be ascribed to greater biological bioavailability of organically complexed minerals than inorganic forms.

In conclusion, using at a much lower levels of organically complexed Cu, Zn and Mn into the broiler diets did not cause to weakness in the plasma antioxidant defence systems and to oxidative damage in liver cell membranes and other cellular tissues. These results therefore indicate the copper, zinc, and manganese from organic sources (Bioplex^{TM}) can be added to broilers diets at much lower levels instead of inorganic forms of those minerals recommend levels by NRC without any negative effect on the antioxidant defense system.

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