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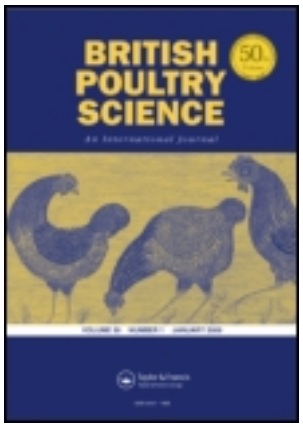

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Effects of organically-complexed minerals on meat quality in chickens

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Effects of organically-complexed minerals on meat quality in chickens

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Abstract 1. The impact of combined lower concentrations of organically-complexed versus inorganic copper, zinc and manganese on meat quality in chickens was investigated.
2. A total of 200 male broiler chicks (Ross-308), 1-d-old and weighing approximately 40 g, were divided into 4 groups comprising three experimental groups and one control group, with each consisting of 50 chicks. All groups were also divided into 5 subgroups, with each containing 10 broiler chicks.
3. All birds were given a starter diet from d 1 to d 21, and a grower diet from d 22 to d 49. The diets were formulated according to the NRC recommendations.
4. The mineral contents of the control diet were supplied using a 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). For the experimental diets, a mineral premix was prepared using organically complexed forms of those minerals at 1/3 (L1), 2/3 (L2) and 3/3 (L3) proportions.
5. After slaughtering and evisceration, the carcases were stored at $3 \pm 0{\cdot}5^{\circ}C$ for 10–12 h, and then the breast fillets removed from the carcases, and stored in a deep-freezer ($-86^{\circ}C$) until analysis (for 30 d). For analyses, the breast muscles were then thawed at 4–6°C for 24 h.
6. The concentration of organically complexed mineral in the diet had no statistically significant effect on pH values of breast fillets. Treatments significantly affected the Thiobarbituric Acid Reactive Substances (TBARS) values.
7. Lightness (L* value) of the fillet from broilers fed the diet containing inorganic minerals was significantly lower than that for broilers fed on the diets containing organically complexed minerals. The redness (a* value) and Chroma (C* value) significantly decreased while Hues (H* value) were higher in the fillet from broilers fed organically complexed minerals compared with those fed inorganic minerals. The yellowness (b* value) was not changed by dietary treatment.

INTRODUCTION

In commercial practice, inorganic trace minerals are used to supply between two and 10 times more minerals than recommended by the National Research Council (NRC) for animal diets (Inal *et al*., 2001; Nollet *et al*., 2007*a*, *b*; Esenbuga *et al*., 2008). These supplemental inorganic trace minerals result in a high level of mineral excretion. This is not only wasteful but is also harmful to the environment (Bao *et al*., 2007). For example, poultry manure applied on a N basis contains Zn and Cu at 660 and 560%, respectively, in excess of crop requirements (Dozier *et al*., 2003). Therefore, nutritionists have started to discuss how to reduce mineral excretion without any negative affect on production performance due to increasing concerns about potential mineral pollution. The use of organically complexed or chelated minerals at

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a much lower concentration for livestock diets has been suggested as an answer to this discussion, based on the hypothesis that such mineral complexes have higher bioavailability than inorganic salt analogues (Bao *et al*., 2007; Nollet *et al*., 2007*a*). It is possible to reduce the excretion of Cu by 75%, Zn by 50%, and Fe by 14% by using organic trace minerals (Bao and Choct, 2009). In recent years, there has been a considerable reduction in the recommended concentrations of organically complexed minerals in broiler diets without any negative effects on broiler performance (Bao *et al*., 2007; Nollet *et al*., 2007*a*; Petrovic *et al*., 2009; Aksu *et al*., 2011), on antioxidant defence systems (Saripinar Aksu *et al*., 2010*a*), or on haematological and biochemical parameters (Saripinar Aksu *et al*., 2010*b*).

On the other hand, it has been widely established that a deficient, as well as an excessive, intake of trace elements could cause oxidative stress (e.g., lipid peroxidation) in poultry (Surai, 2002), but the negative consequences of lipid peroxidation (LPO) can be overcome by adequate dietary supplementation of antioxidants. Cu, Zn, Fe, Mn, and Se are considered antioxidant nutrients—Fe as part of catalase; Cu, Zn, and Mn as part of superoxide dismutase; and Se as part of glutathione peroxidase, and all play interdependent roles in the antioxidant protection of organisms through their termination of LPO reactions. By contrast, the same elements, as transitional metal ions, have the ability to generate highly reactive hydroxyl radicals through their interactions with reactive oxygen species (Aruoma *et al*., 1991). Poultry meat colour is easily influenced by several dietary and environmental variables. As colour references vary from country to country, the colour of poultry meat that is attractive to consumers and how to develop said colours are obviously important factors to be considered by the poultry industry

There is no research on the effect of different concentrations of organically complexed minerals in diets on meat quality, and future research will thus focus on the effects of substitute and reduced mineral diets on meat quality properties. The present study was therefore conducted to evaluate the impacts of various combined concentrations of organic versus inorganic copper, zinc, and manganese on meat quality parameters in broilers.

MATERIALS AND METHODS

Animals, diets, and experimental design

The experiment was carried out in accordance with animal welfare standards and was conducted under the protocols of the Veterinary Faculty of Hatay, Turkey. A total of 200 male broiler chicks (Ross-308), 1-d-old and weighing approximately 40 g, were used in the feeding trial, which lasted until the birds reached 42 d of age. Chicks were randomly allocated to 4 dietary treatments and housed in 20 floor pens (surface area 1 m^2). Each treatment group was divided into 5 subgroups, comprising 10 birds each. The ambient temperature was thermostatically controlled. This temperature was set at 33°C on the first day of the experiment and decreased by 1°C every third day thereafter for the duration of the experimental period. Feed and water were offered *ad libitum*, and lighting was continuous throughout the experimental period. All birds were fed on a starter diet from d 1 to d 21, and a grower diet from d 22 to d 49. Diets were formulated according to NRC (1994) recommendations and analysed by the AOAC (Table 1).

Table 1. *Ingredients and composition of the basal diets*

	Starter phase (d 0 to 21)	Finisher phase (d 21 to 42)
Ingredients, g kg^{-1}		
Maize	515·0	552·0
Wheat	70·0	70·0
Wheat bran	45·0	45·0
Extracted soyabean meal	275·0	240·0
Fish meal	55·0	43·0
Vegetable oil	15·0	25·0
Limestone	10·0	10·0
Dicalcium phosphate	7·5	7·5
Sodium chloride	2·5	2·5
Vitamin-mineral premix*	5·0	5·0
Calculated nutrients		
ME, (MJ kg^{-1})**	12·6	13·0
Crude protein (g kg^{-1})	221	200
Ca, (g kg^{-1})	9·0	8·0
P, (g kg^{-1})	6·0	7·0
Lysine, (g kg^{-1})	11·0	8·0
Analysed minerals non-supplemented feeds		
Cu, (g kg^{-1})	9·63	8·71
Zn, (g kg^{-1})	35·65	30·23
Mn, (g kg^{-1})	38·84	36·63

*: Vitamin premix provides the following per kg: all-trans- retinyl acetate, 1·8 mg; cholecalciferol, 0·025 mg; all-rac-α-tocopherol acetate, 1·25 mg; menadione (menadione sodium bisulphate), 1·1 mg; thiamine (thiamine mononitrate), 1·1 mg; riboflavin, 4·4 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; pyridoxine, 2·2 mg; folic acid, 0·55 mg; cyanocobalamin, 0·02 mg; Fe (from $FeSO_4$), 12·5 mg; I (from KI), 0·3 mg; Se (from NaSe), 0·15 mg.
**ME: Metabolisable energy. The ME, crude protein, calcium, phosphorus and lysine contents were calculated based on their tabular values listed for the feed ingredients (Jurgens, 1996).

The mineral content of the control group diet was controlled using a 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 normal supplementary concentrations and sources of trace minerals in commercial broiler feeds according to the NRC. For the experimental diets, a mineral premix was

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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 kg of diet: Cu, 8·0 mg (as $CuSO_4$); Zn, 40 mg (as $ZnSO_4$); Mn, 60·0 mg (as MnO); Fe, 30·0 mg; I, 2·0 mg; Se, 0·15 mg.
**Organically complexed Mn, Zn and Cu as Bioplex-Mn™, Bioplex-Zn™ and Bioplex-Cu™

prepared using inorganic forms of all components, except for the 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 forms, as Bioplex™ (Table 2). Bioplex containing amino acids from hydrolysed soya protein is an amino acid-hydrate complex bonded with Cu, Zn, and Mn. Bioplex Cu™, Bioplex Zn™, and Bioplex Mn™ contain 100 g/kg of Cu, 150 g/kg of Zn, and 150 g/kg of Mn, respectively (Alltech UK, Standford, England).

Sample collection and measurements

At the end of the trial, the birds were held for 10–12 h without food and water prior to determining their final body weights. Two birds from each subgroup were randomly chosen as having body weights nearest to the average body weight of their own groups, slaughtered via a neck cut, bled for 120s, and semi-scalded 54°C for 30s before mechanical plucking. The birds were eviscerated manually, washed, and allowed to drain for 10 min (Yalcın *et al.*, 1999). After evisceration, the carcases were stored at $3 \pm 0{\cdot}5$°C for 10–12 h and then dissected according to the method utilised by Barbut (2002) and Aksu *et al.* (2008). Then, breast fillets were removed from the carcases, frozen, and stored at −86°C until analysis. Frozen samples were transferred to the meat research laboratory of the food engineering department of Atatürk University under cold chain conditions. Before the analyses, the samples were thawed at 4–6°C for 24 h and then the pH, TBARS, and colour analyses were completed.

Analysis for pH and Thiobarbituric Acid Reactive Substances (TBARS)

The pH values of the samples were determined using a pH meter (SCHOTT L 6880, Lab Star pH). The pH value was measured using a direct probe by thrusting the probe into the breast fillets.

To determine TBARS values, 1 g of ground breast meat sample was taken from each breast fillet of each treatment, and 6 mL TCA solution was added (7·5% TCA, 0·1% EDTA, 0·1% 1-propyl gallate). The mixture was homogenised for 20–30s by Ultra-Turrax and was then filtered over Whatman one. Next, 1 mL 0·02 M ThioBarbituric acid solution was added to the 1 mL filtrate. This mixture was kept in a boiling water bath for 40 min. It was then cooled and centrifuged at 2000 rpm for 5 min. Finally, absorbance was measured at 532 nm (Shimadzu, UV 160), and TBARS values were determined using the standard coefficient as μmol malonaldehyde/kg.

Determination of colour values

A Minolta model colorimeter (CR-200, Minolta Co, Osaka, Japan) was used for the colour measurement of the breast fillet samples, with a white tile as a reference [$L^* = 0$, darkness; $L^* = 100$, lightness (darkness/lightness); a*; $+a^*$ = red, $-a^*$ = green and b*; $+b^*$ = yellow, $-b^*$ = blue; hue $= h^* = \tan^{-1}(b^*/a^*)$; and chroma $= C^* = (a^{*2} + b^{*2})^{1/2}$].

Statistical analysis

The data were subjected to analysis using the general linear model procedure of SPSS (1996) 11·0 for Windows for a completely randomised experimental design. Differences between means were determined by Duncan's multiple range test, at a significance level of $P < 0{\cdot}05$. The model included treatments (control, L-1, L-2, and L-3) as main effects. The results of the statistical analyses are shown in the tables as both mean values ± standard deviations and standard error.

RESULTS

The results of the pH, TBARS, and colour values of the breast fillets obtained when using organically complexed minerals are shown in Table 3. As illustrated, the concentration of dietary organically complexed minerals had no statistically significant effect ($P > 0{\cdot}05$) on the pH values (6·03–6·10) of breast fillets, but the pH values of 3/3 (L3) samples were lower than in the 1/3 (L1), 2/3 (L2), and inorganic samples (Table 3).

In the present study, using much lower concentrations of organically complexed minerals instead of inorganic forms in the broiler diets, significantly affected TBARS values ($P < 0{\cdot}01$), and TBARS values were significantly higher in the 1/3 organic group than in the other groups. The lowest values ($P < 0{\cdot}05$) were found

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Table 3. *The effects of organically complexed minerals on pH, TBARS and colour values of broilers fed on experimental diets*

Parameters	Diets[1]				SEM and significance	
	Control	L1	L2	L3		
pH	6·10 ± 0·12	6·08 ± 0·07	6·10 ± 0·13	6·03 ± 0·09	0·024	NS
TBARS[¥]	14·11 ± 2·48^{b}	22·96 ± 2·13^{a}	12·41 ± 2·19^{c}	13·03 ± 2·65^{bc}	0·530	**
L*	45·43 ± 4·90^{b}	47·51 ± 3·02^{ab}	49·82 ± 3·76^{a}	49·50 ± 4·56^{a}	0·992	**
a*	7·17 ± 1·87^{a}	4·74 ± 1·44^{b}	4·39 ± 1·70^{b}	4·24 ± 1·31^{b}	0·357	**
b*	8·80 ± 1·98	7·78 ± 1·80	8·38 ± 1·21	8·47 ± 2·19	0·409	NS
C*	11·53 ± 1·75^{a}	9·27 ± 1·52^{b}	9·57 ± 1·40^{b}	9·65 ± 1·73^{b}	0·359	**
H*	50·87 ± 10·29^{b}	58·03 ± 10·92^{a}	62·93 ± 9·37^{a}	62·24 ± 11·34^{a}	2·350	**

$^{a–c}$: Means values within a row not having a common superscript are significantly different by least significant differences test ($P < 0·05$). NS: non-significant, *: $P < 0·05$, **: $P < 0·01$, ±: standard deviation of samples.

[¥]: μmol MDA/kg tissue.

[1]Control: Inorganic Cu (as $CuSO_4$), Zn (as $ZnSO_4$) and Mn (as MnO) at NRC recommendation levels.

L1, L2, and L3: Organically complexed Cu, Zn and Mn (as Bioplex^{TM}) at 1/3, 2/3 and 3/3 proportions respectively, instead of inorganic forms of those minerals.

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in the 2/3 organic group, averaging 12·41 ± 2·19 (Table 3).

Table 3 shows the results of the colour characteristics (L*, a*, b*, C*, H*) of the breast fillets of broilers fed experimental diets. Diet treatments significantly affected the lightness, redness, Chroma, and Hues colour values ($P < 0·01$), but the yellowness (b*-value) values were unaffected by dietary treatments. Lightness (L* -value) of the fillets from broilers fed the diet containing inorganic minerals was significantly lower than that of the broilers fed the whole organically complexed mineral diet (Table 3). The redness (a*-value) and Chroma (C*-value) significantly decreased, while Hues (H*-value) were redder in the fillets taken from the broilers fed organically complexed minerals compared to those fed inorganic minerals (Table 3). Yellowness (b*-value) was not changed by dietary treatment, and there were no significant differences in L, a, b, C, and H values between groups fed the different concentrations of organic minerals (Table 3).

DISCUSSION

The pH values obtained from the current study were in the range previously reported for broiler meat (Castellini *et al.*, 2002; Quio *et al.*, 2002; Aksu *et al.*, 2005). There are many factors associated with the interaction between muscle pH and meat quality, such as tenderness, water-holding capacity, cooking loss, juiciness, and microbial stability, and a high correlation between muscle pH and meat colour. A higher muscle pH value is associated with darker meat, whereas lower muscle pH values are associated with lighter meat (Fletcher, 1989).

Research has shown maintenance of the redox balance to be important in ensuring the health of broiler chicks due to their high body lipid contents, which is the cause of lipid peroxidation (Zhang *et al.*, 2008). The major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while copper, zinc, and manganese are essential trace minerals that are important co-factors of SOD and CAT (Haddad *et al.*, 2008). On the other hand, malondialdehyde content in meat is an indicator of lipid peroxidation, and it is most frequently used to measure TBARS values (Shu *et al.*, 1995). In the current study, the degree of fatness observed during slaughter differed among the groups, and the accumulation of subcutaneous, intermuscular, and abdominal fat was higher in carcases from broilers fed the 1/3 level of organically complexed minerals than those fed at the 3/3 level of inorganic and the 2/3 and 3/3 levels of organically complexed minerals. According to our hypothesis, this accumulation probably caused the higher TBARS value found in the breast fillets of the broilers fed at the 1/3 level of organically complexed minerals. On the other hand, the results for the inorganic trace elements showed that the supplementation with inorganic minerals only significantly influenced lipid peroxidation (LPO) by increasing TBARS.

In various studies related to poultry nutrition, researchers have stated that diet composition has significant effects on the TBARS values of breast and drumstick meat (Aksu *et al.*, 2005, 2008). On the other hand, it was also previously determined that some feed additives adversly affect TBARS values after reaching certain levels, but this mechanism could not be elucidated (Aksu *et al.*, 2005). In a previous study (Petrovic *et al.*, 2009), where the effects of dietary supplementation of trace minerals on lipid peroxidation in broiler meat was investigated, it was reported that peroxide and malondialdehyde values increased significantly in the breast and

thigh muscles of chickens fed inorganic minerals, which could possibly be explained by negative interactions between sodium selenite and transition metal ions supplemented in the form of inorganic salts. Petrovic *et al*. (2009) also speculated that the superoxide anion formed during the redox reaction could prevent transition metals of the supplemented inorganic salts from binding to proteins, and instead catalyse the formation of hydroxyl radicals, subsequently increasing LPO. In the current study, the increased TBARS value in the birds fed inorganic minerals can be explained using this statement. On the other hand, the results found in the birds fed 2/3 and 3/3 organically complexed minerals are supported by the findings of Du *et al*. (1996) who reported that negative interactions between the elements declined when their proteinated forms were supplemented into the diet.

Broiler skin and meat colours are affected by various factors, including production management, slaughter, processing, handling, and packaging (Fletcher, 1989; Petracci and Fletcher, 2002). However, as colour preferences vary from country to country, the colour of poultry meat that is attractive to consumers, and the approach to production management, are obviously important for the poultry industry. In addition, poultry meat colour is easily influenced by several dietary and environmental variables. Quio *et al*. (2002) reported that L*, a*, and b* values of fresh breast fillets were 49·6, 3·2, and 4·9, respectively. Castellini *et al*. (2002) found that L*, a*, and b* values were 59·2, 5·0, and 5·2 for breast fillets. Ponsano *et al.* (2004) reported that L*, C*, and h* values were 60·6, 11·25, and 68·5 for breast fillets, respectively.

As far as we are aware, there has been no directly relevant study published on the effects of organically complexed minerals on the colour characteristics of broiler meat. In an indirectly relevant study, Toyomizu *et al.* (2001) observed that spirulina (which is blue-green and is regarded as a rich source of protein, essential amino acids, vitamins, and minerals) resulted in a maximum redness in the breast fillets of chicks when fed a 40 g/kg spirulina diet; while the yellowness of all fillets, including the *Semitendinosus* muscle, increased in a sub-linear fashion with increased spirulina in the diet. In another previous study (Esenbuga *et al*., 2008), where the effects of dietary humate supplementation on performance, slaughter, carcase, and meat colour of broilers were examined, significant improvements were reported in the pH and colour parameters of carcases, breast fillets, and drumsticks when using dietary humate. The L*, a*, b*, H*, and C* values of breast fillets were measured as 59·6, 3·1, 12·9, 72·1, and 13·6, respectively, of broilers fed a diet with 3% supplemental humate.

In conclusion, the use of organic feed ingredients and complexed minerals in animal diets are increasing with the growing interest in organic animal products. Using much lower concentrations of dietary combined organically complexed minerals (Cu, Zn, and Mn) did not affect pH values, but decreased lipid peroxidation of breast fillets and increased the brightness of breast fillets. The results from the present study indicate that future research on adding these minerals to different basal diets will be helpful in understanding both the mechanisms and impacts of organically complexed trace minerals on meat quality.

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