Effect of dietary zinc and sex on broiler breast meat quality, bone marrow color, and blood zinc protoporphyrin to heme ratio (ZPP/H)

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The effects of inorganic dietary zinc (Zn) and sex on broiler breast meat quality, bone marrow color, and blood zinc protoporphyrin to heme ratio (ZPP/H) were studied. A total of 288 d-old male and female Ross 708 chicks were assigned to 0, 120, or 240 mg Zn/kg diets and blood ZPP/H was measured weekly. At 42 d, deboned breast fillets were collected from 3 birds per cage to determine cook yield. Shear force was reduced ($P < 0.05$) in males (0.5 kg) when Zn was added, while there was no effect on female fillets. Dietary Zn had no effect on color of either raw or cooked breast meat while females had lighter (*L) and more yellow (*b**) raw breast color, and less redness (*a**) for both raw and cooked breast meat ($P < 0.05$). Redness of raw marrow measured 1 h after harvest and cooked marrow increased with Zn level ($P < 0.01$). Holding raw bone marrow at room temperature for 1 h reduced *a** by 4 units regardless of diet. Cooked marrow color *a** was reduced by 2.6 units as Zn level increased and was higher in females than males (11.99 vs. 10.89). Dietary Zn had no effect on ZPP/H ratio but females had a higher ratio at 7 d (55.1 vs. 43.4 μmol/mol) and 42 d (37.2 vs. 30.8 μmol/mol) as compared to males. These data indicated that 120 mg Zn/kg diet improved breast fillet texture. Breast color was not affected by Zn but marrow *a** was increased by Zn.**

Keywords: dietary Zn; broilers; meat quality; meat color; meat texture.

Introduction

Zinc is an essential trace element required for many functions, including several enzyme functions (Mills, 1983), nucleic acid metabolism, protein synthesis, carbohydrate metabolism, immune system function, and integrity of cell membranes. Supplementation of trace minerals with large safety margins in broiler chicken diets has resulted in broiler diets with several fold increases in some trace minerals as compared with NRC recommendations (NRC, 1994; Inal et al., 2001; Guclu et al., 2008). Excessive supplementation has led to a high level of mineral excretion into the environment (Skrivan et al., 2006).

In poultry meat, appearance and texture are the two important attributes responsible for initial consumer meat evaluation as well as final product acceptance (Fletcher, 2002). Red and/or bloody discoloration of poultry meat, raw or cooked, has been a chronic yet sporadic problem for the poultry industry. Raw breast meat with red discoloration is objectionable to many customers, and cooked white or dark meat with the red defect is unacceptable to consumers due to the perception that it is undercooked.

No research has been conducted to determine the effects of a diet that is high in zinc and low or normal in iron supplements on the subsequent meat quality of broilers, especially the prevalence of red discoloration in broiler breast meat. High zinc levels in the animal due to dietary supplements could produce elevated ZPP levels in the blood (hemoglobin) and meat (myoglobin), resulting in a red pigment resistant to cooking denaturation even at elevated endpoint temperatures above 185^0F.

Therefore, the aim of this research was to study the effect of different levels of dietary Zn at a fixed Fe level and sex on breast meat quality attributes, bone marrow color, and ZPP/H ratio.

Materials and Methods

Birds and diets. A total of 288 day-old male and female broiler chicks of Ross 344×708 strain were feather-sexed and randomly distributed among 2 battery units with a total of 36 cages. Birds were raised sex-separate with 8 birds per cage. Feed and water were provided for *ad libitum* consumption. Diets were formulated to either meet or exceed the NRC (1994) requirements for broilers except for Zn and Fe. The hypothesis was based on the assumption that zinc (Zn) will replace the iron (Fe) in hemoglobin and myoglobin when Fe is deficient in the feed thus affecting the color of the meat. To test the hypothesis, feed ingredients were selected to contain very low amounts of Zn and Fe to reduce the influence of any organically available Zn and Fe in the feed ingredients. One basal diet was formulated without including mineral premix. The three experimental diets were formulated from the same basal diet to ensure that birds had access to same nutrients. Inorganic zinc sulfate ($ZnSO_4$) was added at the same level for both starter and grower to achieve a Zn concentration in the experimental diets of either: 0, 120, or 240 mg Zn/kg diet while maintaining Fe at a fixed level (111 and 110 mg/kg for starter and grower diets, respectively).

Meat quality measurements. At 42 d of age, 3 birds per cage were withdrawn from feed for 12 h, then electrically stunned for 11 s, killed by exsanguination, and allowed to bleed for 90 s. Birds were then scalded at 55°C for 90 s in a rotary scalder, picked for 30 s in a drum picker, and manually eviscerated. Carcasses were chilled overnight by immersion in ice chilled water, and manually deboned the following day. Femur bones were collected from the thighs.

1. Cook yield. Breast fillets (*Pectoralis major*) were weighed, placed on aluminum pans, and cooked in a forced air oven. Fillets were cooked to an internal temperature of 75°C (approximately 25 min), as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe. The cooked fillets were cooled to room temperature and weighed again to determine cook yield as percentage of the cooked weight relative to the raw weight.

2. Shear force. Cooked breast fillets samples were tested for texture using a Warner-Bratzler shear device (Warner-Bratzler meat shear, Bodine Electric Company, Chicago, USA). Two samples per breast fillets (2×2×2 cm) were sheared in a direction perpendicular to the muscle fiber. The maximum force measured when cutting the samples was expressed in kg.

3.Color. Color was measured on the medial side of both raw and cooked breast fillets. Color was measured by the CIE L*a*b* system using a Minolta Chroma Meter CR-400. The colorimeter was calibrated using white tile (reference number 13033071; Y = 93.9, x = 0.3156, y = 0.3318). Triplicate measurements were taken for each sample. CIE lightness (*L**), redness (*a**), and yellowness (*b**) were measured.

4. Red discoloration induction. This test was conducted according to the procedure described by Smith and Northcutt (2004b). Collected femurs were pooled by treatment and sex. Bony marrow was collected by removing the exterior bone from the epiphyseal end caps, and the porous hard bone marrow was collected, manually minced using mortar and pestle until it turned into a thick granular paste. Boneless, skinless broiler breast fillets were obtained fresh (refrigerated) from a local retail store. External fat was trimmed and connective tissue removed. Cleaned meat was chopped manually and blended for 30 s in a food processor. Glass tubes open at both ends were used (OD = 20 mm, ID = 17 mm × 200 mm length). For each tube, 10 g of minced meat was divided into 2 equal parts. One part meat was put into the tube at one end, followed by 1 g of minced bony marrow, then the second part of the meat and each tube was sealed with a #2 rubber stopper. Three replicate tubes were prepared for each treatment × sex interaction. Tubes were placed in a circulating water bath set at 95°C. Tubes were removed immediately when temperature reached 75°C (approximately 2 min) as measured by a Therma Plus thermocouple with a 125-cm hard-wired temperature probe. Tubes were placed in an ice water bath until the temperature reached 4°C (approximately 45 min). Stoppers were removed from the glass tubes and the cooked plugs were pushed out of the tubes (plug diameter = 16 mm). Plugs were separated manually into 2 parts at the contact surface with bony marrow; the excess

marrow was cleaned off the meat surface. CIE lightness (*L**), redness (*a**), and yellowness (*b**) were measured at the contact surface of the meat with bony marrow in replicate.

5. Bony marrow color. CIE color for lightness (*L**), redness (*a**), and yellowness (*b**) of raw bony marrow paste was measured immediately and 1 h after harvest. Samples were then cooked following the procedure described above. Cooked bony marrow color was measured in replicate.

Blood zinc protoporphyrin to heme ratio. To determine the ZPP/H ratio baseline 10 males and 10 females were killed after hatching and blood samples were collected from the heart using non-heparinized syringes. The ZPP/H concentration in whole blood was measured immediately using a hematofluorometer (Aviv hematofluorometer Model 206D, Aviv Biomedical, NJ, USA). The instrument measured the ratio of ZPP fluorescence to heme absorption. A drop of whole blood was placed on a microscope cover glass for insertion into the ZPP hematofluorometer and fluorescence was measured in μmol/mol heme with an assumed hematocrit of 42%. Blood samples were collected from one bird per cage weekly. Blood samples were collected from the heart up to 21 d, after that, blood samples were collected from the wing via the brachial vein.

Statistical analysis. Data were analyzed as a two-way ANOVA using the GLM procedure of SAS (SAS version 9.2, SAS Institute, Cary, NC, USA, 2008). The model tested the main effects of dietary Zn and sex, as well as their interaction. Differences between means were separated using the LS means method and significance was reported at a probability level of $P \leq 0.05$. The experimental unit for statistical analysis of measured parameters was the cage.

Results and discussion

Cook yield and texture. Dietary Zn had no effect on cook yield of breast fillets and values were 77.5, 75.9, and 76.8 % for dietary Zn supplemented at 0, 120, and 240 mg/kg, respectively. Sex also had no effect on cook yield and values were 77.2 and 76.3 % for males and females, respectively. Warner-Bratzler shear force values were not affected by either dietary Zn or sex. Shear force values were 2.75, 2.77, and 2.68 kg for dietary Zn supplemented at 0, 120, and 240 mg/kg, respectively. Male and female shear force values were 2.74 and 2.73, respectively. Salim et al. (2012) investigated the effect of adding 25 mg/kg organic Zn on meat texture of male and female broilers and reported that Zn had no effect on texture but males had higher shear force values. Salakova et al. (2009) reported that males had more tender meat as compared to females. However, in the current study, the response of males and females to dietary Zn differed according to the level supplemented.

Color. CIE lightness (*L**), redness (*a**), and yellowness (*b**) were measured for raw and cooked breast fillets, and cooked breast meat with results presented in Table 1. Adding different levels of dietary Zn did not affect the color of the breast fillets (data not shown). Raw breast fillets harvested from females were lighter, less red, and more yellow as compared to males ($P < 0.05$). These results were consistent with the findings of Salakova et al. (2009). Values were for *L** 50.20 and 54.02, *a** 2.36 and 1.98, and *b** 5.78 and 6.83, for males and females, respectively. However, when color was measured on the cooked breast fillets, there was no difference between males and females. Table 2 presents the result of breast meat cooked in contact with bony marrow. The raw breast meat used in red discoloration initially measured 64.71, 2.87, and 12.42 for *L**, *a**, and *b**, respectively. Lightness (*L**) of cooked breast meat with bony marrow tended to be higher in females, while *b** was comparable among males and females. Redness (*a**) of cooked breast meat with bony marrow was higher than *a** of raw and cooked breast fillets, with males having higher *a** as compared to females. Values were 19.42 and 15.71 for males and females, respectively. Salakova et al. (2009) reported that cooked breast meat was significantly lighter, redder and more yellow than the raw meat, which was consistent with our findings of breast meat color. The effect of dietary Zn on breast meat color has not been adequately studied.

Table 1. Effect of sex on breast meat color.

Sex	*L**	*a**	*b**

Raw breast fillet color			
Male	50.20[b]	2.36[a]	5.78[b]
Female	54.02[a]	1.98[b]	6.83[a]
SEM[1]	0.5	0.1	0.2
P-value	0.001	0.05	0.001
Cooked breast fillet color			
Male	78.07	3.35	16.37
Female	79.46	3.30	16.83
SEM[1]	0.6	0.1	0.3
P-value	0.120	0.777	0.212

[a,b]Means in a column that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Standard error of mean (SEM) for n=54 fillets.

Table 2. Effect of sex on color of breast meat cooked in contact with bony marrow.

Sex	*L**	*a**	*b**
Male	49.98	19.42[a]	14.25
Female	52.45	15.71[b]	13.65
SEM[2]	0.9	1.0	0.8
P-value	0.06	0.02	0.587

[a,b]Means in a column that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error of mean (SEM) for n=9.

Bony marrow color results are presented in Table 3. Lightness (*L**) of raw bony marrow measured immediately after harvest increased ($P < 0.05$) when Zn was added to the diet, however *a** and *b** were not affected by dietary Zn. When color was measured 1 h after harvest, all color coordinates were decreased, and the decrease in *L** was larger when Zn was supplemented to the diets. Values for *L** measured immediately after harvest were 38.63, 40.75, and 39.34, and values measured 1 h after harvest were 38.50, 38.33, and 35.81 for dietary Zn of 0, 120, and 240 mg Zn/kg, respectively. Redness (*a**) and *b** were increased when dietary Zn was supplemented. The same trend for *L** remained after cooking, where *L** was reduced in cooked bony marrow by dietary Zn supplementation. Redness (*a**) and *b** were reduced by cooking, and *a** was the lowest when dietary Zn was supplemented at 120 mg/kg. Yellowness of cooked bony marrow (*b**) was also reduced by adding dietary Zn. Females had lighter, more red, and more yellow color as compared to the males when raw bony marrow color was measured immediately after harvest, however, only *L** remained higher ($P < 0.05$) in females when color was measured 1 h after harvest. However, when bony marrow was cooked, all color coordinates measured remained higher in females. Cooked bony marrow color values were for *L** 38.58 and 40.35, *a** 10.89 and 11.99, and *b** 7.35 and 8.18, for males and females, respectively.

Smith and Northcutt (2003) reported that approximately 11% of selected fully-cooked retail chicken products evaluated for bloody/red defect were affected. One cause for this discoloration was determined to be bony marrow (Smith and Northcutt, 2004a). In the current research, we were unable to detect the effect of high dietary Zn on breast meat color. However, the effect was apparent for bony marrow color.

Table 3. Effect of dietary Zn and sex on bone marrow color.

Variable	Dietary Zn (mg/kg)			SEM[3]	Sex		SEM[2]	P-value	
	0	120	240		Male	Female		Zn	Sex
Raw marrow color immediately after harvest									
*L**	38.63[b]	40.75[a]	39.34[ab]	0.5	38.76[b]	40.39[a]	0.4	0.05	0.02
*a**	32.31	35.53	34.50	1.0	32.51[b]	35.72[a]	0.8	0.147	0.03
*b**	14.56	16.69	15.44	0.6	14.10[b]	17.03[a]	0.5	0.145	0.01
Raw marrow color 1 h after harvest									
*L**	38.50[a]	38.33[a]	35.81[b]	0.4	35.79[b]	39.30[a]	0.4	0.01	0.001
*a**	27.91[c]	31.79[a]	29.88[b]	0.3	30.10	29.62	0.3	0.001	0.258
*b**	11.14[b]	12.94[a]	12.18[a]	0.2	12.28	11.89	0.2	0.01	0.217
Cooked marrow color									
*L**	39.99[a]	40.10[a]	38.30[b]	0.4	38.58[b]	40.35[a]	0.3	0.002	0.001

*a**	12.59[a]	10.00[b]	11.78[a]	0.4	10.89[b]	11.99[a]	0.3	0.001	0.01
*b**	7.98[a]	7.90[a]	7.42[b]	0.1	7.35[b]	8.18[a]	0.1	0.02	0.001

[a,b]Means in a row within each main effect that possess different superscripts differ significantly ($P \leq 0.05$).
[2]Standard error of mean (SEM) for n=9.
[3]Standard error of mean (SEM) for n=6.

Blood zinc protoporphyrin to heme ratio. Blood ZPP/H ratio was measured weekly (Table 4). To establish a baseline of ZPP/H ratio, a total of 10 males and 10 females were bled. Average ZPP/H ratio in the males was 37.2 µmol/mol, and for females 47.1 µmol/mol. Dietary Zn had no effect on ZPP/H ratio (data not shown). However, females had higher ($P < 0.05$) ZPP/H ratio at 7 and 42 d, and tended ($P < 0.1$) to be higher in females at 21 d.

High ZPP/H ratio indicated that Zn had replaced Fe in the heme molecule, thus changing the color of hemoglobin and myoglobin. ZPP/H ratio has generally been used as a screening test to detect Fe deficiency and lead poisoning. It has been used with ducks to test for lead poisoning (Roscoe and Nielsen, 1979).

The stable bright red color of Italian Parma ham was believed to be a result of Zn-protoporphyrin complex that was produced during the maturation process. This complex has been found to be very stable and does not change with either heat or light exposure (Wakamatsu et al., 2004). The mechanism has remained unknown. However, it has been suggested that since Zn was the second most abundant cation in skeletal muscles (Kagawa, 2001) that it replaced the Fe in the heme, which resulted in the stable bright red color. We were unable to detect this effect in broiler breast meat, possibility due to low myoglobin content of chicken breast muscle as compared to red meat (Young and West, 2001), or the Zn level in the feed was not sufficient to induce the red color in breast meat.

Numata and Wakamatsu (2009) conducted a study to summarize the causes of natural red pigments in food and food products and found that during long curing process zinc present in the tissue replaced the iron in myoglobin and any remaining hemoglobin. The resulting zinc protoporphyrin molecule (ZPP) was a very heat stable red pigment. This process has now been patented for producing red pigments for food use that were heat stable (Numata and Wakamatsu, 2009).

Table 4. Effect of sex on blood zinc protoporphyrin to heme ratio (ZPP/H).

	Age (d)					
Sex	**7**	**14**	**21**	**28**	**35**	**42**
ZPP/H (µmol/mol)						
Male	43.4[b]	48.0	33.6	32.0	29.8	30.8[b]
Female	55.1[a]	49.2	38.9	36.3	34.2	37.2[a]
SEM[4]	2.4	3.2	1.9	1.8	1.9	2.0
P-value	0.001	0.799	0.07	0.101	0.109	0.04

[a,b]Means in a column that possess different superscripts differ significantly ($P \leq 0.05$).
[4]Standard error of mean (SEM) for n=18.

Conclusions

Results of the study indicated that 120 mg Zn/kg diet improved breast fillet texture. Breast color was not affected by Zn although bone marrow *a** was increased by Zn. Males and females differed in their meat quality characteristics and response to mineral supplementation. Further research will be required to fully delineate the effect of high Zn on the formation of ZPP and red color in broiler breast meat.

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