Effect of different levels of dietary zinc, manganese, and copper from organic or inorganic sources on performance, bacterial chondronecrosis, intramuscular collagen characteristics, and occurrence of meat quality defects of broiler chickens

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ABSTRACT The aim of the experiment was to assess the effects of 2 dietary levels of trace minerals (TM) zinc, manganese, and copper either from organic (OTM) or inorganic (ITM) sources on broiler performance, carcass traits, intramuscular collagen (IMC) properties, occurrence of hock burns (HB), foot pad dermatitis (FPD), femoral and tibia head necrosis, and breast muscle abnormalities (white striping, WS; wooden breast, WB; poor cohesion, PC). A total of 3,600 one-day-old male chicks were randomly assigned to one of 4 dietary treatments in a 2 × 2 factorial arrangement (9 replicates of 100 birds/dietary treatment). Birds were slaughtered at 31 (thinning) and 51 d of age. Body weight, daily weight gain (DWG), feed intake, feed conversion rate (FCR), and mortality were determined. A significant effect of the source of TM supplementation was found only in 51-day-old chickens. Birds of the OTM groups were heavier ($P < 0.05$), with a higher ($P < 0.05$) DWG and a better FCR ($P < 0.05$) compared with those of the ITM groups. OTM significantly reduced the lesion scores of femoral head ($P = 0.004$) and total leg (femur + tibia, $P = 0.02$) compared to ITM, which is mainly caused by the reduction of the percentages of femoral head transitional degeneration (FHT, $P = 0.04$) and femoral head necrosis (FHN, $P = 0.07$). Carcass traits were similar among the experimental groups. No alleviating effect of TM administration on the incidence of FPD and HB in 31- and 51-day-old chickens was found. Similarly, the occurrence and the degrees of WS and WB in 51-day-old chickens was affected neither by the doses nor by the source of TM supplied. IMC characteristics of broiler pectoral muscle were not affected ($P > 0.05$) by the different sources and doses of TM administrated.

Key words: broiler chicken, organic and inorganic trace minerals, foot dermatitis, muscle abnormalities, bacterial chondronecrosis

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INTRODUCTION

Bird nutrition is focused on the main dietary components (protein and energy) and mineral supplements, from both inorganic and organic sources, that are routinely incorporated into concentrate feeds to satisfy animal nutritional requirements (López-Alonso, 2012; Świątkiewicz et al., 2014). The presence of trace minerals (**TM**) in animal feed is vital for the animal's metabolic processes (Soetan et al., 2010). TM, such as zinc (**Zn**), manganese (**Mn**), and copper (**Cu**) are essential to maintain health and productivity in broiler chickens and in all livestock animals. In fact, they are catalysts or constituents of several enzymatic systems, and they are part of hundreds of proteins and organic molecules involved in intermediary metabolism, hormone secretion pathways, and immune defense systems (Dieck et al., 2003); as a result, they influence growth, bone development, feathering, enzyme structure and function, and appetite of broiler chickens (Nollet et al., 2007). Despite the fact that the role of TM in animal health is well established, most of the feedstuffs do not contain adequate amounts of various TM according to the requirements that change during the rapid growth and development of the animal and the production cycle (Bao and Choct, 2009; López-Alonso, 2012). Traditionally, in commercial poultry diets, minerals are supplemented in the form of inorganic salts, such as carbonates, oxides, or sulphate to provide the levels of minerals satisfying the birds' requirements, as suggested by various feeding standards. However, inorganic trace minerals (**ITM**) can suffer from high rates of loss due to dietary antagonism, which causes a significant reduction in their bioavailability (Saripinar-Aksu

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et al., 2012). As a consequence, feed manufacturers use much higher doses of minerals that often exceed the birds' requirements (Świątkiewicz et al., 2014). The continuous use of these inorganic mineral salts as feed additives has been implicated in the environmental mineral pollution arising from their accumulation on poultry wastes (Bao and Choct, 2009). Nevertheless, due to the increasing concerns about potential mineral pollution, the use of organically complexed or chelated minerals at much lower concentration has been suggested for livestock diets, based on the hypothesis that such mineral complexes have a higher bioavailability than inorganic salt analogues (reviewed in Saripinar-Aksu et al., 2012). Organic mineral sources exist in the form of metal amino acid chelates and metal proteinates. Abdallah et al. (2009) reported that chicks fed diets containing 100% organic minerals (Zn, Cu, Mn, and Fe) had higher BW and better feed conversion in comparison with those fed diets with inorganic minerals. Similar results were obtained by El-Husseiny et al. (2012). Differently, Nollet et al. (2007) and Peric et al. (2007) observed no significant differences between birds fed inorganic and organic minerals as for productive performance.

Collagen is the major structural protein of internal tissues, including cartilage, bone, and muscle. It has been established that collagen plays a key role in determining meat toughness of different domestic animals, including birds (Maiorano et al., 2012). Raw chicken meat is generally very soft and, when cooked, it can even be cohesive. Nevertheless, a new emerging quality issue in fast-growing birds is the poor cohesion of meat (tendency toward separation of muscle fiber bundles), which has been related to immaturity of intramuscular connective tissue; it is believed that genetic progress has put more stress on the growing bird (Petracci and Cavani, 2012) and, consequently, poultry meat is tender, but may turn fragile, even mushy (Puolanne and Voutila, 2009), and cooked chicken breast meat is generally fragmented (Maiorano et al., 2012; Petracci and Cavani, 2012). Moreover, in recent years, some studies indicate an increasing occurrence of new quality abnormalities in the breast muscles of broiler chickens (Mazzoni et al., 2015), as well as an increase of hock and foot pad dermatitis (**FPD**) (Shepherd and Fairchild, 2010) and femoral head necrosis (McNamee and Smyth, 2000). Although it is supposed that these abnormalities are due mainly to genetic factors, nutritional approaches are investigated in the attempt to alleviate their incidence (Petracci and Cavani, 2012). Femoral head necrosis, now called bacterial chondronecrosis with osteomyelitis (**BCO**), is the most common cause of lameness in commercial broilers (Wideman et al., 2012). It is supposed that BCO is initiated by microtrauma to poorly mineralized columns of cartilage cells in the proximal growth plates of the leg bones, followed by colonization of opportunistic bacteria translocation via systemic blood flow (Wideman and Prisby, 2013). Jiang et al. (2015) identified that certain bacterial subgroups are preferentially selected in association with the development of BCO lesions.

Zn has been shown to have an effect on DNA replication and can influence directly the immune function. Moreover, Zn plays a role in other different aspects of poultry production, including bone and skin establishment (reviewed in Peric et al., 2007), and it is involved in the synthesis of collagen and keratin (Pardo and Selman, 2005; Richards et al., 2010). Cu acts as a cofactor of lysyl-oxidase, the enzyme that controls cross-linking of collagen and elastin (Kagan and Wande, 2003). Mn is a cofactor of hydrolase, decarboxylase, and transferase enzymes (Murray et al., 2000). It is involved in the formation of glycosaminoglycans containing chondroitine sulphate (Beattie and Avenell, 1992) and also in the synthesis of proteoglycans present in bone growth plate of poultry species (Liu et al., 1994), and it is essential for the maintenance of bone mineralization (Strause et al., 1986).

TM play a very important role in bone and skeletal development. Zn promotes collagen synthesis and turnover in cartilage and developing bone (Caterson et al., 2000; Krane and Inada, 2008). Cu is essential for cross-linking of collagen and elastin, and an adequate Cu intake is crucial to obtain peak bone mass in long bones and to prevent femur fractures (Heaney, 1988; Miggiano and Gagliardi, 2005). Mn is an essential element for bone and tendon development as its deficiency causes tibial twisting and bending and tibiometatarsal joint enlargement and malformation, resulting in slippage of the gastrocnemius tendon from its condyles (Underwood and Suttle, 1999). Mn is also associated with shorter and thicker long bones in poultry (Underwood and Suttle, 1999). Therefore, Zn, Cu, and Mn are supplemented in livestock diets either as ITM or OTM or a combination of both. We hypothesized that supplementation of TM could improve bone mineralization and structure integrity, which could reduce bacteria translocation, thereby alleviating BCO lesions.

The aim of the present experiment was to evaluate the effect of low or high supplementation of inorganic and organic sources of Zn, Cu, and Mn in diet on in vivo performance, carcass traits, intramuscular collagen (**IMC**) characteristics, lesion score of femoral and tibial head necrosis, occurrence of FPD, white striping (**WS**), wooden breast (**WB**), and poor cohesion in the pectoral muscle of broiler chickens.

MATERIALS AND METHODS

Birds, Diets, and Experimental Design

A total of 3,600 one-day-old male chicks (Ross 308) were hatched from the same breeder flock and supplied by a commercial hatchery. The chicks were housed in a poultry house divided in 2 boxes, each composed by 18 floor pens of 6 m^2 each. Pens were equipped with pan feeders to assure at least 2 cm/bird of front

Table 1. Composition and calculated analysis diets (as is basis).

	STARTER (0 to 14 d)	GROWER (15 to 30 d)	FINISHER (31 to 51 d)
Ingredients, g/kg			
Corn	421.7	349.6	127.3
White corn	–	–	150.0
Wheat	100.0	200.0	250.1
Sorghum	–	–	50.0
Soybean, extracted meal	231.1	206.3	176.0
Expanded soybean	100.0	100.0	130.0
Sunflower	30.0	30.0	30.0
Corn gluten	40.0	30.0	–
Vegetable oil	30.8	44.3	54.8
Dicalcium phosphate	15.2	12.0	5.70
Calcium carbonate	9.10	6.50	5.20
Salt	2.70	2.70	2.50
Sodium bicarbonate	1.50	1.00	1.50
Coline chloride	1.00	1.00	1.00
Lysine sulphate	5.90	5.50	4.60
DL-methionine	2.70	2.90	3.00
Threonine	1.50	1.40	1.40
Xylanase[1]	0.80	0.80	0.80
Phytase[2]	1.00	1.00	1.00
Vitamin-mineral premix[3]	5.00	5.00	5.00
Calculated (analyzed) composition (g/kg)			
Dry matter	885.7 (886.4)	886.5 (887.2)	886.4 (887.1)
Crude protein	227.0 (229.3)	214.9 (215.6)	197.4 (201.1)
Crude fat	70.6 (71.3)	82.4 (82.9)	97.4 (97.4)
Fiber	30.8	30.4	30.7
Ash	58.5 (59.1)	51.7 (52.1)	44.9 (45.6)
Threonine	9.80	9.10	8.50
Arginine	14.2	13.4	12.7
Isoleucine	9.30	8.70	8.00
Lysine	13.8	12.9	12.1
Methionine	6.70	6.20	5.90
Methionine+cystine	10.3	9.70	9.10
Tryptophan	2.60	2.50	2.40
Valine	10.4	9.80	9.00
K	9.30	8.90	8.90
Na	1.60	1.40	1.50
Cl	2.30	2.30	2.20
Ca	9.10	8.00	5.90
P	6.30	5.70	4.60
ME (Kcal/kg)	3.076	3.168	3.264

[1] Roxazyme G2, Basel, Switzerland.

[2] Phyzime XP 500, Danisco, Copenhagen, Denmark.

[3] Provided the following per kg of diet: vitamin A (retinyl acetate), 13,000 IU; vitamin D3 (cholecalciferol), 4,000 IU; vitamin E (DL-α_tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6.0 mg; pantothenic acid, 6.0 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B_{12} 20 μg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

space and an automatic drinking system (one nipple/10 birds). Bedding material was chopped wheat straw (2 kg/m^2). At 31 d of age, when the stocking density was approximately 27 kg BW/m^2, a portion of the flock (40 birds/pen) was slaughtered (thinning), while the remaining birds were grown up till 51 d old, when a stocking density of about 39 kg BW/m^2 was reached. Temperature was gradually decreased from 32°C on d zero to 20°C on d 30 and was kept constant thereafter. The lighting program was: 23L:1D in the first wk, 18L:6D from d 8 to d 48, and 23L:1D from d 49 to slaughter. The experiment consisted of 4 different dietary treatments that were assigned to broilers in a completely randomized design. There were 9 replicates of 100 chicks per each dietary treatment. The experimental design was a 2 × 2 factorial arrangement including 2 TM sources of Zn, Mn, and Cu, organic (**OTM**) and ITM, and 2 doses, low (**LO**) and high (**HI**). The ITM supplementation was ZnO, MnO, and CuSO4. The OTM supplementation consisted of a combination of 3 chelates of metals and DL-methionine hydroxy analogue (Mintrex, Novus). To a common basal diet, divided in starter (zero to 14 d), grower (15 to 30 d), and finisher (31 to 51 d), formulated without the inclusion of Zn, Mn, and Cu in the mineral vitamin premix, ITM and OTM were added (Table 1). The inclusion levels for both ITM and OTM were as follow: High doses of Zn and Mn were 60-50-50 mg/kg feed, whereas Cu was 15-12.5-12.5 mg/kg, respectively, for starter, grower, and finisher diets. Low doses of Zn and Mn were 40-32-32 mg/kg feed, whereas Cu was 10-8-8 mg/kg, respectively, for starter, grower, and finisher diets. The analyzed content of minerals in the diets are reported in Table 2. Since Zn, Mn, and Cu requirements for broiler chickens are

Table 2. Zinc, manganese, and copper content for either organic (OTM) or inorganic (ITM) experimental diets.

			TM content, mg/kg feed		
Feeding phase	TM sources	Dosage	Zn	Cu	Mn
Starter	OTM	Hi	97	22	85
Starter	OTM	Lo	77	17	65
Starter	ITM	Hi	92	20	89
Starter	ITM	Lo	72	19	68
Grower	OTM	Hi	91	19	83
Grower	OTM	Lo	73	15	65
Grower	ITM	Hi	96	13	88
Grower	ITM	Lo	79	20	71
Finisher	OTM	Hi	93	19	87
Finisher	OTM	Lo	75	15	69
Finisher	ITM	Hi	99	14	81
Finisher	ITM	Lo	69	10	61

controversial and vary noticeably among authors and breeding companies' recommendations (NRC, 1994; Leeson and Summer, 2001; Aviagen 2014), in the present work, we considered as reference values those mostly adopted in current broiler diets.

Representative samples of all diets were collected for dry matter (method 934.01; AOAC International, 2000), crude protein (method 990.03; AOAC International, 2000), crude fat (method 954.02; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), and minerals (Zn, Cu, and Mn) analysis by inductively coupled plasma optical emission spectrometry - Optima 2100 (PerkinElmer, Waltham, MA) (method 985.01; AOAC International, 2000).

To increase the challenging conditions in terms of nitrogen excretion and impact on the incidence of FPD, a feeding plan of 3 phases, rather than the 4 usually adopted in commercial condition, was chosen (Table 1). The experimental diets were supplied in mash form. Feed and water were provided for ad libitum consumption. Birds were handled according to the principles stated in EU Directive 63/2010 (European Union, 2010) regarding the protection of animals used for experimental and other scientific purposes.

Performance

At zero, 14, 31 (thinning), and 51 (slaughter) d birds were counted and weighed on a pen basis. At the same intervals, feed orts were weighed pen wise. Daily weight gain (**DWG**), feed intake (**FI**), feed conversion rate (**FCR**), and mortality were calculated for each feeding phase and for the overall experimental period. FI and FCR were corrected for mortality, taking into account weight and life duration of dead birds.

Slaughter Surveys

For the thinning procedure, 40 birds/pen were slaughtered at the approximate BW of 1.6 kg (31 d old); while, the remaining birds were slaughtered in a commercial abattoir at about 3.9 kg (51 d old). Birds were weighed (after a fasting period of 12 h) and electrically stunned (120 V, 200 Hz). After evisceration the carcasses were air-chilled (pre-cooling at 5°C for 60 min, followed by chilling at 0°C for 90 min). Cold carcass weight was recorded (at 24 h at 4°C), and carcass yield percentage was calculated. The incidence of any visible external carcass defects, such as FPD and hock burns (**HB**), were evaluated on all the processed birds (31 and 51 d old). FPD were measured by collecting one foot per each processed birds and scored (0 = no lesion; 1 = mild lesion, < 0.8 cm; 2 = severe lesion), according to the classification of Ekstrand et al. (1998). The lesion score was calculated applying the formula reported in the EU proposal COM 221 (Commission of the European Communities, 2005): the number of feet from class 0 did not contribute to the score; the number of feet from class 1 was multiplied by 0.5, the feet from class 2 were multiplied by 2, and those scores were added; the total was divided by the sample size and multiplied by 100. At 24 h postmortem, carcasses from birds 51 d old were dissected (breast, legs, and wings and their percentages were calculated based on cold carcass weight). After breast deboning, the occurrence of WS, WB, and poor cohesion defects were evaluated on all processed birds slaughter at 51 d old. Fillets showing no striping were classified as normal (score 0), whereas for those muscles with WS appearance, it was determined whether the defect level was moderate (white striation < 1 mm; score 1) or severe (white striation > 1 mm; score 2) based on the intensity and thickness of white striation according to the criteria proposed by Kuttappan et al. (2012). The detection of WB was conducted macroscopically evaluating the shape, consistency, and color, as well as presence and type of exudate and/or hemorrhages (Sihvo et al., 2014). Fillets showing no defects were scored 0, whereas those presenting moderate hardened areas along with moderate WS were score 1, and those with extended hardened areas along with severe WS were scored 2. The defect of poor cohesiveness of breast muscle was evaluated by assigning score 0 to normal fillets; score 1 to fillets that presented moderately loose in structure, perceivable by pinching the muscle in the cranial surface; and score 2 to fillets with extended

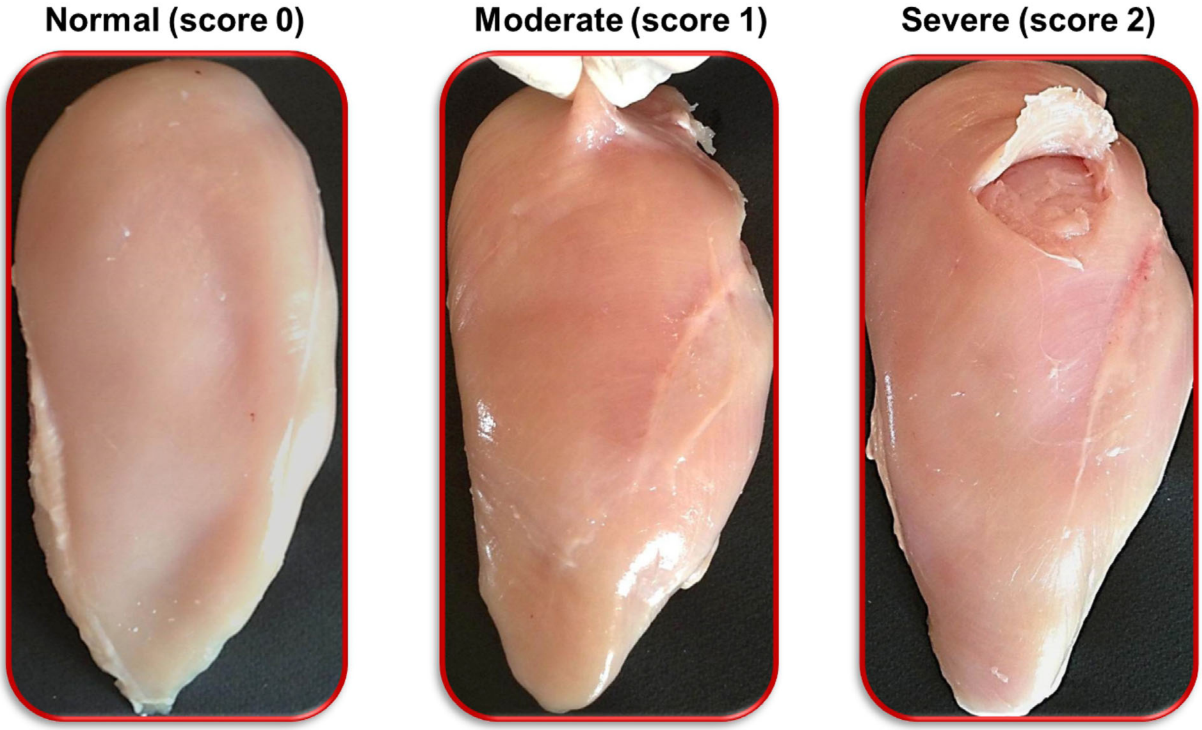

Figure 1. Poor cohesion classification of broiler breast meat: normal breast fillet (no poor cohesion, score 0); fillet with moderate loose structure that is perceivable by pinching the muscle in the cranial surface (score 1); and fillet with extended superficial lacerations (score 2).

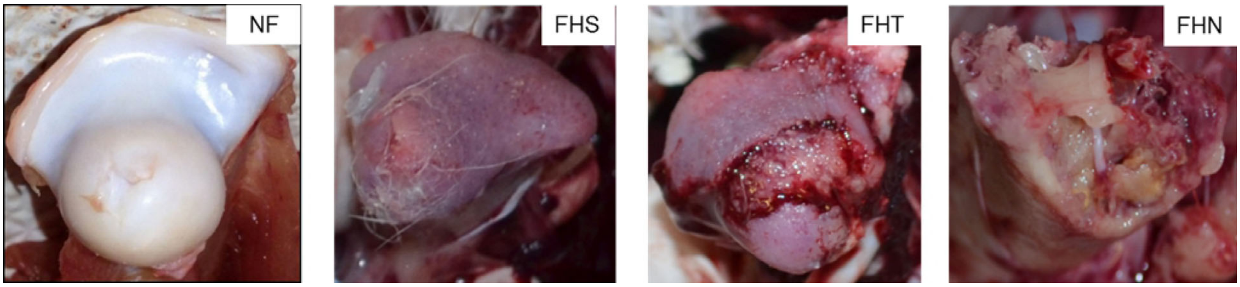

Figure 2. Macroscopical appearance of femoral heads. NF: normal femur without apparent abnormalities; FHS: femoral head separation with loss of white cartilage cap; FHT: femoral head transitional degeneration with progressive necrosis and fracturing of growth plate; FHN: femoral head necrosis with perforation, fracturing, and necrosis/osteomyelitis.

superficial lacerations (Figure 1). After the chilling phase, 27 carcasses per experimental group (3 birds per pen) were collected; after discarding very anomalous samples affected by Oregon disease, severe white striping and extreme PSE-like condition, 18 breast fillets (P. major muscles)/group were vacuum packaged and stored frozen at approximately −40°C pending IMC analysis.

Femoral and Tibia Head Necrosis Evaluation

At farm, one non-lame bird and 2 lame birds per pen were randomly selected, labeled, and then collected at the slaughterhouse for the evaluation of bacterial chondronecrosis with osteomyelitis (**BCO**) lesions as described by Wideman et al., 2012 with modifications. The proximal femoral heads were evaluated and scored as described below. Score 0: macroscopically normal femur (**NF**) without apparent abnormalities; Score 1: femoral head separation (**FHS**); Score 2: femoral head transitional (**FHT**) degeneration with progressive necrosis, ulceration, erosion, and fracturing of the growth plate; Score 3: femoral head necrosis (**FHN**) with perforation, fracturing, and necrosis/osteomyelitis on femoral head (Figure 2). The proximal tibial heads were evaluated and scored as below: Score 0: normal tibial (**NT**) head with struts of trabecular bone in the metaphyseal zone fully supporting the growth plate; Score 1: mild proximal tibial head necrosis (**THN**) with necrotic voids in the metaphyseal zone undermining the support of the growth plate; Score 2: severe THN (**THNs**) in which the growth plate was imminently threatened or damaged; Score 3: caseous THN

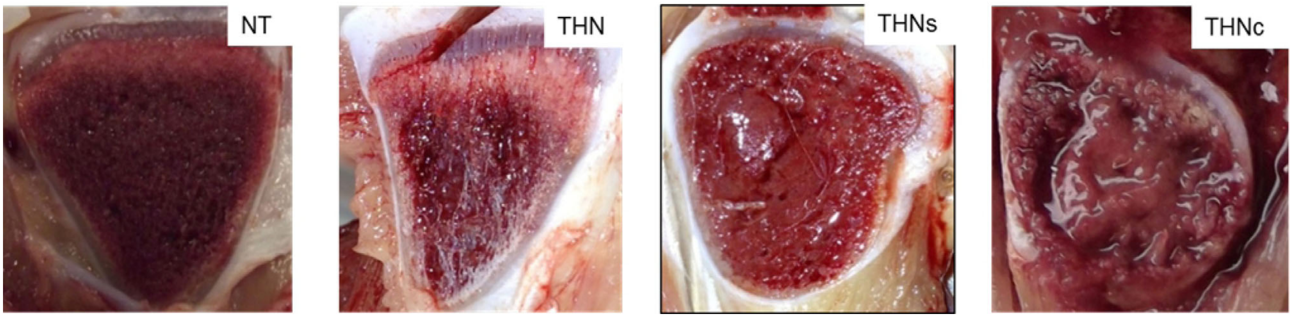

Figure 3. Macroscopical appearance of tibia heads section. NT: metaphyseal trabecular bone fully supports growth plate; THN: tibial head necrosis, necrotic void in metaphyseal trabecular bone; THNs: severe tibial head necrosis, severe necrotic void endangers growth plate; THNc: severe tibial head necrosis with bacteria colonies caseous exudate providing macroscopic evidence of bacterial infection.

(**THNc**) in which caseous exudates or bacterial sequestrae were macroscopically evident (Figure 3).

Collagen Analysis

Muscle samples were thawed just prior to analysis at room temperature trimmed of fat and epimysium, lyophilized for 48 h, weighed, and hydrolyzed in Duran tubes in 5 mL 6-N HCl at 110°C for 18 to 20 h for determination of hydroxyproline (Woessner, 1961) and crosslinking. The analyses were carried out in duplicate. Intramuscular collagen concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. Hydroxylysylpyridinoline (**HLP**) concentration, the principal non-reducible crosslink of muscle collagen and highly correlated with the thermal stability of collagen (McCormick, 1999), was determined using the procedure reported by Maiorano et al. (2012). A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Luna C18 column (250 × 4.6 mm × 5 μm; Phenomenex, Torrance, CA), was used. The concentration of HLP residues in the samples was calculated based on the concentration of collagen in each hydrolyzate, assuming that the molecular weight of collagen was 300,000 and the molar fluorescence yield of pyridoxamine (internal standard) was 3.1 times that of HLP. Crosslink concentration was expressed as moles of HLP per mole of collagen.

Statistical Analyses

Data were evaluated by 2 × 2 factorial ANOVA. Pen was the experimental unit for growth, FI, FCR, and BCO lesions. As for BCO lesions, lameness also was used as a factor for data analysis as there was dramatic difference in BCO lesions between non-lame and lame birds. Each individual bird was considered as the experimental unit for carcass characteristics, breast muscle defect, FPD, HB, and IMC properties. In addition, collagen amount and crosslinks in white-striped fillets and normal fillets were evaluated by two-way ANOVA with interactions, while data regarding carcass yield and incidence of meat abnormalities were analyzed using chi-square test. Data analysis was performed using package SAS (SAS Institute, 1988).

RESULTS AND DISCUSSION

Performance

The effects of source and dose of TM on productive performance of 31- and 51-day-old chickens are shown in Table 3. At thinning (31 d) none of the performance traits (BW, DWG, FI, FCR) was affected ($P > 0.05$) by the different source and dose of TM administration. No effect was observed on growth performance after replacing the inorganic source with low levels of organic TM in broiler diets during a 39-day trial period (Nollet et al., 2007), as well as with different doses of organic TM on broiler performance and mortality during a 42-day trial period (Nollet et al., 2008). In contrast, Yi et al. (2007) in a 21-day growth assay on Cobb 500 male chicks found a linear increase in cumulative gain:feed ratio for Mintrex Zn, Cu, and Mn; authors also observed for Mintrex Zn and Mn a linear increase in cumulative gain increasing Mintrex supplementation. However, the dosages adopted in the trials by Yi et al. (2007) were higher than those used in the present experiment; moreover, the high dose was 4 folds higher than the lower one. In our study, the lack of dose-related effect could be due to the fact that even lower levels of TM administration can meet the chicken dietary requirements. In fact, both levels (HI and LO) of inclusion of TM in the feed fulfilled the NRC recommendations (Cu, 8 mg/kg; Zn, 40 mg/kg; Mn, 60 mg/kg), but are lower than those recommended in the commercial practice for Ross 308 (Aviagen, 2014).

At 51 d of age, chickens of the OTM group were heavier (+ 2%; $P < 0.05$), due to the higher ($P < 0.05$) DWG, and they had a better FCR ($P < 0.05$) in comparison to those of the ITM group; FI was not affected ($P < 0.05$) by the source. These results are in agreement with Yuan et al. (2011) who reported that supplementation with 100% Mintrex-Zn/Mn

Table 3. Effect of source and dose of TM on productive traits of broilers at 31 and 51 d of age.

	Source (S)		Dose (D)			*P*-value		
Item[1]	OTM[2]	ITM[2]	HI[3]	LO[3]	SEM	S	D	S*D
31 d								
Pens (n.)	16	16	16	16				
Final BW (g)	1,579	1,567	1,579	1,567	8.94	0.40	0.39	0.80
DWG (g/bird/d)	51.1	50.6	51.0	50.7	0.29	0.23	0.46	0.56
FI (g/bird/d)	2,480	2,464	2,468	2,475	0.31	0.24	0.64	0.99
FCR (g/bird/d)	1.615	1.619	1.610	1.624	0.01	0.69	0.11	0.52
Mortality (%)	0.89	1.89	1.47	1.41	0.01	0.09	0.77	0.03
51 d								
Pens (n.)	16	16	16	16				
Final BW (g)	3,953	3,872	3,921	3,899	20.08	0.01	0.46	0.80
DWG (g/bird/d)	77.5	75.9	76.9	76.4	0.39	0.01	0.45	0.79
FI (g/bird/d)	6,756	6,729	6,734	6,750	0.41	0.50	0.71	0.75
FCR (g/bird/d)	1.727	1.753	1.733	1.749	0.01	0.03	0.17	0.50
Mortality (%)	1.45	2.68	1.88	2.18	0.02	0.10	0.98	0.05

[1] DWG = daily weight gain; FI = feed intake; FCR = feed conversion ratio.
[2] OTM = organic trace mineral; ITM = inorganic trace mineral.
[3] HI = high dose; LO = low dose.

significantly improved the DWG and decreased fecal mineral excretion compared to inorganic supplementation, while 80% of Mintrex-Zn/Mn admistration did not affect significantly the growth performance. Differently, El-Husseiny et al. (2012) reported that the partial replacement (50%) of inorganic sources of Zn (ZnO), Mn (MnO), and Cu ($CuSO_4$) with organic forms of these microelements improved growth performance, carcass characteristics, and excreta in 35-day-old Arbor Acres female broiler chickens. As was also observed for 31-day-old chickens, no significant dose-effect ($P > 0.05$) of TM administration was found for the performance traits (BW, DWG, FI, FCR) in 51-day-old chickens. Mortality was affected neither by source nor dose of TM administration. However, significant interactions ($P = 0.05$) between source and dose of TM administration were observed for mortality (OTM-LO = 1.00%; OTM-HI = 1.88%; ITM-LO = 3.22%; ITM-HI = 1.88%). Similarly, in a study conducted on 54-day-old broilers, Manangi et al. (2012) found no significant differences in chicken mortality between ITM (Zn, 100 ppm; Cu, 125 ppm; Mn, 90 ppm) and OTM (Zn, 32 ppm; Cu, 8 ppm; Mn, 32 ppm) groups.

Slaughter Performance

The carcass and cut-up yields obtained from birds slaughtered at 31 and 51 d of age are shown in Table 4. None of the carcass traits was affected by the different source and dose of TM administration. Indeed, the carcass traits obtained from chickens fed lower doses of TM, both organic and inorganic forms, were comparable to those of chickens fed higher doses of TM. El-Husseiny et al. (2012) found that 35-day-old female broiler chickens (Arbor Acres breed) fed a diet supplemented with 50% of the broiler strain recommendation of organic Zn, Mn, and Cu had an increase in carcass and breast muscle yields, compared to birds fed ITM or OTM at different levels. Differently, Zhao et al. (2010) reported that the reduction of one half of the standard grow-out program (Zn, 80 ppm; Cu, 8 ppm; Mn, 120 ppm, as sulfates), containing 50:50 of inorganic and chelated forms, had no effect on carcass traits of 52-day-old male and female Ross 308, while, an increase of carcass weight was found only on female Cobb 700 supplemented OTM. The discrepancy in the above mentioned findings could be attributed to different factors including genetics, sex (Zhao et al., 2010), age, environment, and nutrition (Bao and Choct, 2009).

Table 4. Effect of source and dose of TM on carcass traits of broilers at 31 and 51 d of age.

	Source (S)		Dose (D)	
Item	OTM[1]	ITM[1]	HI[2]	LO[2]
31 d				
n.	720	720	720	720
Carcass yield (%)	71.4	71.4	71.3	71.5
Chi-square	0.000		0.001	
51 d				
n.	953	930	949	934
Carcass yield (%)	71.5	71.5	71.6	71.4
Chi Square	0.000		0.001	
Breast yield (%)	31.4	31.4	31.3	31.5
Legs yield (%)	41.8	42.2	42.0	42.0
Wings (%)	19.8	19.7	19.7	19.7
Chi-square	0.02		0.001	

[1] OTM = organic trace mineral; ITM = inorganic trace mineral.
[2] HI = high dose; LO = low dose.

Foot Pad Dermatitis

Foot health has been an important component of broiler welfare and of the economy of broiler production. Foot pad dermatitis is a condition that causes necrotic lesions on the plantar surface of the foot pads in growing broilers and turkeys and it is affected by several factors such as litter moisture and type, stocking density, and ventilation rate (Meluzzi et al., 2008b; Meluzzi and Sirri, 2009). The HB is a type of contact

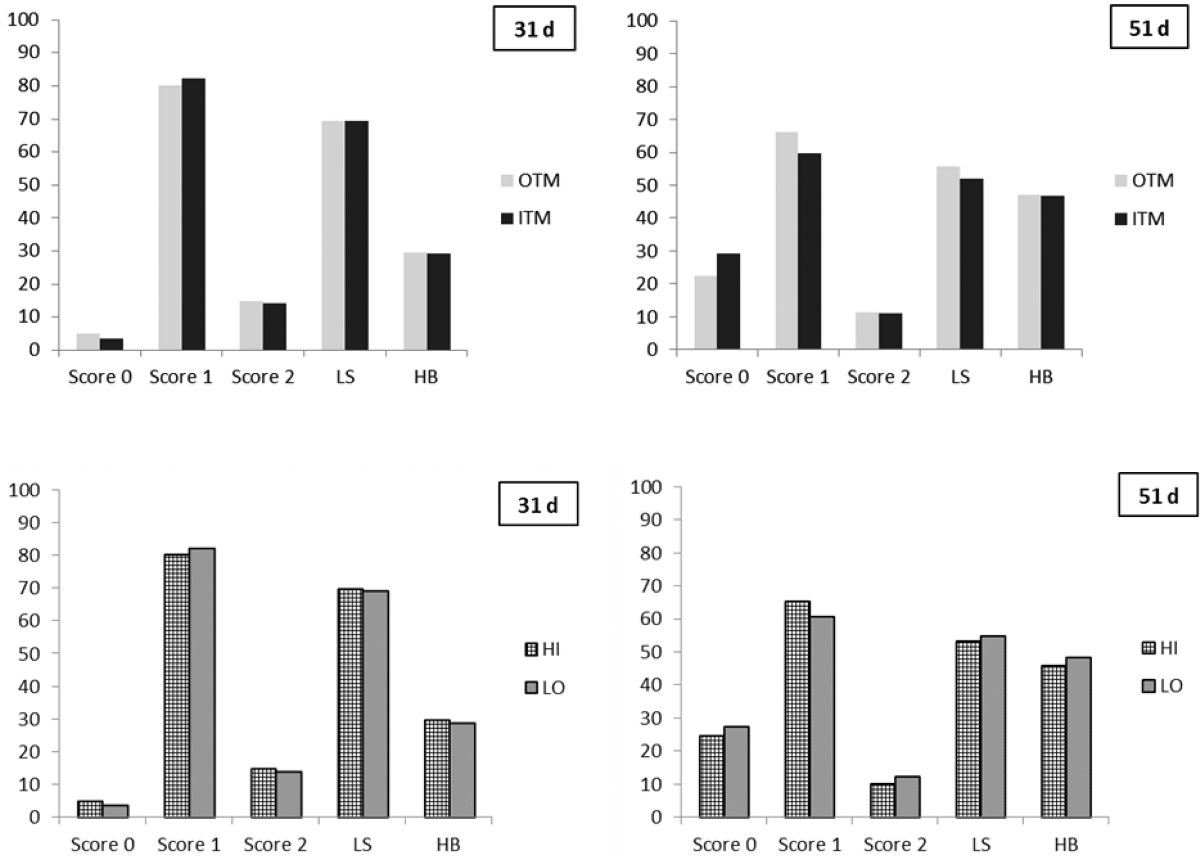

Figure 4. Incidence (%) of foot pad lesions (Score 0, 1, and 2), lesion score (LS) and hock burn (HB) in relation to the trace mineral source (OTM and ITM) and to the doses (HI and LO) evaluated at 31 and 51 d.

dermatitis, an ulceration of the skin whereby affected areas show brown or black discoloration as well as inflammation and necrosis (Greene et al., 1985), and seems to be positively correlated with FPD (Meluzzi et al., 2008a, Meluzzi and Sirri, 2009). The incidence of FPD and HB recorded at slaughter for 31- and 51-day-old chickens was affected neither by the doses nor by the source of TM supplied (Figure 4). At 31 d in all the experimental groups the percentage of birds without FPD lesions (score 0) was extremely low (ranging from 3.4 to 5.1%). On the contrary, higher values of mild (ranging from 80.2 to 82.4%) and severe lesions (ranging from 13.9 to 14.7%) were recorded. The total lesion score ranged from 69.1 to 69.5, which appeared considerably higher than the threshold (=50) reported in the COM proposal 221 (Commission of the European Communities, 2005). The high lesion score could be mainly due to the challenging rearing conditions (high CP levels and high stocking density) selected to focus on potential preventive effects of the source and dose of TM administration on the onset of FPD. This was an unexpected result because literature reports that TM administration may alleviate the incidence of FPD as Zn and Cu play a key role in both physiological and pathological tissue remodeling (Richards et al., 2010; Zhao et al., 2010). At 51 d the incidence of FPD lesions ranged from 22.4 to 29.3% (score 0), from 59.6 to 66.3% (score 1), and from 10.2 to 12.1% (score 2). The total lesion score ranged from 52.1 to 55.6. The higher incidence of birds without FPD recorded at 51 d, compared to that of the younger birds, is mainly attributable to the healing process of feet that may occur when, after thinning, litter conditions improve due to the reduced stocking density. In agreement with our results, Vieira et al. (2013) found an improvement of the footpad integrity in 41-day-old broilers compared to those slaughtered at 21 d-old, reared in challenging conditions; however, they also evidenced a reduction of the incidence of FPD in the groups fed diets containing organic Zn compared to those fed inorganic Zn. The incidence of HB was more evident in 51-day-old chickens (ranging from 45.7 to 48.4%) than those of 31-day-old (ranging from 28.9 to 29.7%). Incidence of HB lesion is influenced by BW of the birds, with heavier birds being more prone to lying on litter, as well as by poor litter quality (Shepherd and Fairchild, 2010; Kapell et al., 2012), age, and genotype (Ask, 2010).

Femoral and Tibia Head Necrosis Evaluation

Femoral and tibial head lesion scores, the total leg lesion scores (femoral lesion score + tibial lesion score),

Table 5. Effect of source and dose of TM on the incidence of femoral, tibial and total leg (femoral+tibial lesions) lesion scores in lame and non-lame chickens at 51 d of age.

	Source (S)		Dose (D)		Lame (L)			*P*-value			
Item	OTM[1]	ITM[1]	HI[2]	LO[2]	NL[3]	LA[3]	SEM	S	D	L	S*D
Femoral head											
n.	54	54	54	54	36	72					
Lesion score	0.86	1.18	1.13	0.92	0.56	1.49	0.077	0.004	0.06	<0.0001	0.04
Tibial head											
n.	54	54	54	54	36	72					
Lesion score	0.21	0.29	0.26	0.24	0.03	0.47	0.066	0.38	0.77	<0.0001	1.00
Total leg (Femoral + Tibial)											
n.	54	54	54	54	36	72					
Lesion score	1.07	1.47	1.39	1.15	0.58	1.96	0.120	0.02	0.17	<0.0001	0.57

[1] OTM = organic trace mineral; ITM = inorganic trace mineral.
[2] HI = high dose; LO = low dose.
[3] NL = non-lame birds; LA = lame birds.

Table 6. Effect of source, dose, and lameness on the percentages of BCO lesion categories of broilers at 51 d of age.

							S*L								
	Source (S)		Dose (D)		Lame (L)		NL		LA			*P* value			
Item[1]	OTM[2]	ITM[2]	HI[3]	LO[3]	NL[4]	LA[4]	OTM	ITM	OTM	ITM	SEM	S	D	L	L*S
NF	26.4	19.4	19.4	26.4	41.7	4.2	50.0	33.3	2.8	5.6	6.4	0.44	0.44	<0.0001	0.28
FHS	58.3	50.0	51.4	56.9	58.3	50.0	50.0	66.7	66.7	33.3	7.3	0.42	0.59	0.42	0.02
FHT	15.3	27.8	26.4	16.7	0.0	43.1	0.0	0.0	30.6	55.6	4.3	0.04	0.11	<0.0001	0.04
FHN	0.0	5.6	5.6	0.0	0.0	5.6	0.0	0.0	0.0	11.1	2.1	0.07	0.07	0.07	0.07
FHS+FHT+FHN	73.6	83.3	83.3	73.6	58.3	98.6	50.0	66.7	97.2	100.0	6.2	0.27	0.27	<0.0001	0.43
NT	75.0	75.0	70.8	79.2	94.4	55.6	94.4	94.4	55.6	55.6	5.9	1.00	0.32	<0.0001	1.00
THN	23.6	20.8	26.4	18.1	5.6	38.9	5.6	5.6	41.7	36.1	5.3	0.71	0.27	<0.0001	0.71
THNs	1.4	4.2	2.8	2.8	0.0	5.6	0.0	0.0	2.8	8.3	1.9	0.31	1.00	0.04	0.31
THNc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	–	–
THN+THNs+THNc	25.0	25.0	29.2	20.8	5.6	44.4	5.6	5.6	44.4	44.4	5.9	1.00	0.32	<0.0001	1.00

[1] NF = normal femoral head; FHS = femoral head separation; FHT = femoral head transitional degeneration; FHN = femoral head necrosis; NT = normal tibial head; THN = mild proximal tibial head necrosis; THNs = severe THN; THNc = caseous THN with macroscopically evident caseous exudates or bacterial sequestrae.
[2] OTM = organic trace mineral; ITM = inorganic trace mineral.
[3] HI = high dose; LO = low dose.
[4] NL = non-lame birds; LA = lame birds.

and percentages of each lesion category were compared among treatments.

Lame birds had much more severe femoral, tibial, and total leg lesions (Table 5) and higher percentages of femoral and tibial head lesions (Table 6) than non-lame birds, indicating that the lameness of broilers in this trial was mainly caused by BCO lesions. Low levels of TM tended to reduce the femoral head lesion scores (P = 0.06, Table 5) and the percentage of FHN (P = 0.07, Table 6) compared to high levels of TM. OTM significantly reduced femoral head (P = 0.004) and total leg lesion scores (P = 0.02) compared to ITM (Table 5), which is due to the increase of NF percentage in non-lame birds, the increase of FHS percentage (P = 0.02), and reduction of FHT (P = 0.04) and FHN percentages (P = 0.07) in lame birds (Table 6). Dibner et al. (2007) state that fast-growing birds may need higher levels and/or more bioavailable forms of minerals to obtain optimal bone development and joint health. OTM have been shown to be more bioavailable than ITM (Paik et al., 1999; Cao et al., 2000; Guo et al., 2001; Yan and Waldroup, 2006; Wang et al., 2007).

We hypothize that the higher bioavailability of OTM could be more effective than ITM in providing enough trace minerals for bone mineralization and structure integrity, which could reduce opportunistic bacteria translocation via systemic blood flow, therefore alleviating femoral and tibial head lesions.

Pectoral Muscle Abnormalities

The incidence and degrees of breast myopathies (WS, WB, and poor cohesion) are reported in Table 7. Neither the source nor the dose of TM administered affected the incidence and the degrees of WS. The total incidence of moderate and severe white striped breast fillets ranged from 60 to 61.1% (moderate: from 21.5 to 22.8%; severe: from 37.8 to 39.0%) among the 4 experimental groups. The incidence of severe WS was 37.8

Table 7. Effect of source and dose of TM on Pectoral muscle abnormalities of broilers at 51 d of age.

	Sources (S)		Dose (D)	
Item	OTM[1]	ITM[1]	HI[2]	LO[2]
n.	400	400	400	400
White striping				
Score 0 (no lesions) (%)	39.6	39.3	38.8	40.0
Score 1 (mild lesions) (%)	22.6	21.6	22.8	21.5
Score 2 (severe lesions) (%)	37.8	39.0	38.3	38.5
Chi-square	0.134		0.223	
Wooden breast				
Score 0 (no lesions) (%)	88.8	84.3	87.0	86.0
Score 1 (mild lesions) (%)	0.80	1.50	1.30	1.00
Score 2 (severe lesions) (%)	10.5	14.3	11.8	13.0
Chi-square	3.741		0.387	
Poor cohesion				
Score 0 (no lesions) (%)	53.1	64.5	57.4	60.3
Score 1 (mild lesions) (%)	37.6	31.5	36.3	32.8
Score 2 (severe lesions) (%)	9.30	4.00	6.30	7.00
Chi-square	14.909***		1.185	

[1] OTM = organic trace mineral; ITM = inorganic trace mineral.
[2] HI = high dose; LO = low dose.
***: $P < 0.001$.

and 39.0% in OTM and ITM, respectively; moreover, similar WS values were observed for the TM doses. No significant differences were also found for the incidence and degrees of WB among all the experimental groups. However, fillets affected by WB were of much lower extent compared to the fillets affected by WS, ranging from 11.3 to 15.8% (moderate: from 0.8 to 1.50%; severe: from 10.5 to 14.3%). In fact, it has been proposed that WS occurs during an early stage of muscle degeneration, whereas breast fillets become "wooden" only at a later stage of development (Mudalal et al., 2015). Nevertheless, even if no consistent trend was observed, some variation was seen in the frequency of breast myopathies. Chickens fed OTM showed a slightly lower incidence of WB compared to those fed ITM (10.5 vs 14.3%, respectively). Regarding the doses, the higher one seems to reduce the incidence of severe lesions (11.8 vs 13.0%, respectively); however, these differences were not statistically significant. On the other hand, chickens fed OTM seem more prone to exhibit a higher percentage of fillets with poor cohesion ($P < 0.01$) compared with those of the ITM group. The total incidence of mild and severe poor cohesion ranged from 35.5 to 46.9% (mild: from 31.5 to 37.6%; severe: 4.0 to 9.3%) among all groups. However, there was no strong evidence to indicate that a different source and dose of TM administration would reduce the incidence of pectoral muscle abnormalities. On the other hand, no information is available from current literature on the effect of source and dose of TM administration on the occurrence of breast muscle abnormalities in chickens. In general, the magnitude of breast myopathies found in the present study was rather high, confirming that breast fillets from heavy-sized broilers (live weight 3.9 kg) are nevertheless recognized to be the market class that is more prone to exhibit a higher incidence of myopathic lesions and meat abnormalities (Lorenzi et al., 2014; Mazzoni et al., 2015).

IMC Properties

IMC characteristics of broiler pectoral muscle were not affected ($P > 0.05$) by the different source and dose of TM administration (Table 8), even though it is known that zinc is crucial in the synthesis of collagen and keratin (Pardo and Selman, 2005; Richards et al., 2010) and copper plays a role in the cross-linking of collagen and elastin (Kagan and Wande, 2003). The lack of source- and dose-related effects found in the present study could be due to the fact that even lower levels of TM administration fulfilled the chicken dietary requirements. Nevertheless, no information is available from current literature on the effect of TM administration on IMC properties in chickens.

The association of IMC characteristics with respect to the occurrence of WS (data not shown) has revealed a slightly higher ($P = 0.093$) content of IMC in white-striped fillets compared to the normal ones (24.10 vs. 21.80 μg/mg, respectively), while, the degree of collagen maturation was not affected ($P = 0.599$) by the occurrence of WS (0.134 vs 0.122 mol/mol of collagen, for normal and white-striped fillets, respectively). These findings are consistent with those of Mudalal et al. (2015) and Petracci et al. (2014) who found a higher total collagen content in white-striped fillets compared to normal fillets; they observed also a reduction of the total amount of protein and an increase of intramuscular fat in white-striped fillets. As suggested by Petracci et al. (2014), the changes in chemical composition of white-striped fillets can be associated with the occurrence of a degeneration process for muscle fibers,

Table 8. Effect of source and dose of TM on IMC properties of broilers at 51 d of age.

	Source (S)		Dose (D)			*Effect*		
Item[3]	OTM[1]	ITM[1]	HI[2]	LO[2]	SEM	*S*	*D*	*S*D*
n.	18	18	18	18				
IMC (μg/mg[4])	22.39	21.92	21.59	22.72	0.54	0.659	0.294	0.733
HLP (mol/mol of collagen)	0.131	0.130	0.124	0.136	0.009	0.942	0.498	0.949

[1] OTM = organic trace mineral; ITM = inorganic trace mineral.
[2] HI = high dose; LO = low dose.
[3] ICM = intramuscular collagen; HLP = hydroxylysylpyridoline.
[4] of lyophilized muscular tissue.

which can lead also to lipidosis and fibrosis (higher collagen content) in fillets affected by WS. Differently, Mazzoni et al. (2015) did not find any significant difference in collagen content among the 3 muscle degeneration degrees (mild, moderate, severe) even if severely myodegenerated samples were characterized by a strong decrease of total protein content accompanied by a thickening connective tissue layers at perimysium level.

In conclusion, under the condition of the current study, the supplementation with OTM significantly improved growth and FCR and reduced femoral head necrosis in 51-day-old broilers, but did not affect carcass traits. Hock burns and foot pad conditions in 31- and 51-day-old chickens were not affected by the treatments. The incidence of breast myopathies was rather high and no alleviating effect of TM administration was found. The IMC properties of breast muscle were similar among groups. However, there was found a slightly higher content of IMC in white-striped fillets compared to the normal ones. In light of these results, we believe that the replacement of ITM with OTM could have a possible advantage in productive performance and a potential reduction of mineral excretion, mitigating the detrimental effect of intensive poultry production on the environment. However further research is warranted to confirm these remarks and to better assess the real requirements in TM for modern broiler strains, and also to elucidate the effects of TM on the occurrence of emerging breast myopathies, since no information is available from the current literature.

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