Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.020

RESEARCH ARTICLE

Assessment of Serum Trace Elements in Thiram Induced Tibial Dyschondroplasia Chickens

Houqiang Luo[1,§*], Kun Li[2, §], Sheji Weng[3], Yu Bai[1], Hui Zhang[2], Khalid Mehmood[2,4], Muhammad Shahzad[4] and Jiaxiang Wang[5*]

[1]College of Animal Science, Wenzhou Vocational College of Science and Technology, Wenzhou 325006, People's Republic of China; [2] College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People's Republic of China; [3]Hospital of Wenzhou Medical University, 109 Xueyuan XiRoad, Wenzhou 325027, Zhejiang, China; [4]University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur, 63100 Pakistan; [5]College of Animal Science, Yangtze University, Jingzhou 434023, People's Republic of China
*Corresponding author: chviolet1984@sina.com (HQL); wangjiaxiang1109@163.com (JXW)

ARTICLE HISTORY (17-264)

Received: July 26, 2017
Revised: November 28, 2017
Accepted: December 02, 2017
Published online: January 20, 2018

Key words:
Blood
Broiler
Deficiency
Feed supplement
Serum
Thiram

ABSTRACT

This study was conducted to determine the concentrations of various trace elements in thiram induced tibial dyschondroplasia chickens. A total 30 day-old male chicks equally divided into two groups including thiram group (n=15) and control group (n=15). Control group was given basal diet throughout the study. Tibial dyschondroplasia was induced by adding thiram @ 50 mg/kg into basal diet from day 3 post-hatch till the end of experiment. Two milliliter bloods were collected from ten chickens of each group on day 7 and 14. The serum was separated by centrifugation and blood serum was assessed in triplicates by Agilent 7700x equipped with an ASX 250 auto sampler. The results were analyzed by student's t-test, which revealed that the concentration of Cr, Mn, Fe, Co, Ni, Mo, Cd and Pb in thiram group were increased significantly ($P<0.05$), while the concentration of Cu in was significantly decreased on day 10 compared to control group. The concentrations of Cr, Mn, Fe, Ni and Pb in thiram groups on day 14 were significantly lower than control group, while concentration of Mo was significantly higher in thiram group. The concentrations of Co, Cu and Cd on day 14 were non-significantly different in both groups. The current study shows that thiram effect Cr, Mn, Fe, Co, Ni, Mo, Cd, Pb and Cu in the chick body significantly which is not a good for chicken health. So, it provides considerations to take the effective measures for poultry feed supplement to prevent the occurrence of TD.

To Cite This Article: Luo H, Li K, Weng S, Bai Y, Zhang H, Mehmood K, Shahzad M and Wang J, 2018. Assessment of serum trace elements in thiram induced tibial dyschondroplasia chickens. Pak Vet J, 38(1): 101-104. http://dx.doi.org/10.29261/pakvetj/2018.020

INTRODUCTION

Tibial dyschondroplasia (TD) is a leg disorder estimated approximately 30% in many avian species and causes significant economic losses to poultry industry word-wide (Shahzad *et al*., 2014). There are several theories that put forward to explain its etiology but actual mechanism of TD development is not known (Leach and Monsonego-Ornan, 2007). In post hatch poultry, thiram is the most common and accurate source to produce TD (Rasaputra *et al*., 2013). Tetramethyl-thiuram disulfide (thiram) is a dithiocarbamate organic compound, which is commonly utilized as fungicide and pesticide in agriculture (Shahzad *et al*., 2014). Thiram exerts its cytotoxic effects by membrane damage, inhibition of glutathione metabolism, mitochondrial injury, cell death, and inhibition of angiogenesis (Shahzad *et al*., 2014).

TD is characterized by the accumulation of prehypertrophic chondrocytes which make avascular and non-mineralize cartilage in the tibial growth plate (Shahzad *et al*., 2014). TD cause lameness, and bone breakage, which are harmful to animal welfare (Shahzad *et al*., 2014). Previous studies demonstrated that the incidence of TD is affected by the calcium/phosphorus ratio, and copper-deficient diets (Leach and Monsonego-Ornan, 2007). Trace elements play important role in the body including maintain fluid balance, promote blood, and bone development and nervous system (Hostetler *et al*., 2003). So, mineral plays an important role during the incidence of TD because trace elements are involved in

[§]These authors contributed equally to this study.

many biological mechanisms, such as superoxide dismutases, electron transfer, and antioxidant defense (Beede, 2006). Essential trace elements including cobalt (Co), copper (Cu), manganese (Mn), zinc (Zn), selenium (Se), iron (Fe), Chromium (Cr), cadmium (Cd), and molybdenum (Mo) have important impact on productive performances in chickens. Previously no study was conducted on serum trace elements, so scarce information is available about serum trace elements in thiram induced TD chickens. Keeping in view the importance of trace elements in birds, and diseases caused due to fluctuation in the concentrations of these elements, the present study was designed to determine the concentrations of Ni (nickel), Cu, Mo, Cd, Pb (plumbum), Zn, Se, Ti (titanium), V (vanadium), Cr, Mn, Fe, Co, and As (arsenic) in thiram induced tibial dyschondroplasia Chickens.

MATERIALS AND METHODS

Ethics approval: The animal experiments were conducted after the approval of institutional Animal Welfare and Research, Ethics Committee guideline of Huazhong Agricultural University (Wuhan, China).

Animals and study design: Day-old male broiler chicks (n=30) were raised under standard hygienic conditions at temperature 93°F, and humidity 60%. The two groups including thiram group (n=15) and control group (n=15). Control group was given basal diet throughout the study. TD was induced by thiram (50 mg/kg in feed in dietary) from day 3 post-hatch. The current study protocol and dose of thiram was according to previous study (Shahzad *et al*., 2014). Briefly, all the chickens were offered based diet and water *ad libitum* till day 3 post hatch. After that on day 4 post hatch thiram group chickens were exposed to TD by adding thiram @ 50 mg per kg of feed, while control group was raised on basal diet till the end of experiment. Two milliliter blood was collected from ten chickens on day 7 and 14 from each group by cardiac puncture. Blood samples were centrifuged at 3000×g for 20min for separation of blood serum and stored at -70°C, until subsequent use and further analysis.

Trace elements assessment: The trace elements in serum samples were measured by employing ICP-MS (inductively coupled plasma mass spectrometry). The presence of artificial correlations was detected by several possible routes of detection standard concentrations, examining triplicate of each sample and taking average value, through considering the pre-analytical factors like contamination by two elements at the same time and instrumental shortcomings (isotopic or polyatomic mass interferences) (Barany *et al.*, 2002). Agilent 7700x equipped with an ASX 250 auto sampler was used to perform the analysis. The system operation was maintained at a radio frequency power of 1550W, argon (Ar) plasma gas flow rate of 15L/min; and 1L/min and 0.99L/min Ar auxiliary and carrier gas flowrate, respectively. For measurements, 21× dilutions of samples were prepared in 1%HNO3 in acid-treated 15mL conical tubes with an 8 mm sampling depth and 0.35 mL/min sample uptake rate for at least 24h. Data were quantified with external standards for Cr, Mn, Fe, Co, Ni, Cu, Mo, Cd, Pb, Zn, Se, Ti, V and As in 1% HNO3. The internal standard consisting of 103Rh was added manually to all solutions. For each sample, data were acquired in triplicate and then average was taken.

Statistical analysis: The standard concentrations in the calibration line were analyzed to confirm the instrument validation (the lowest and the highest ones). The axis cut point of the calibration line was utilized to acquire the detection limits. Comparison between the Mean±SE (standard error) values of control and thiram groups was carried out by Student's t-test and oneway ANOVA followed by Turkey's honest test for continuous variables by piloting SPSS (20.0) with $P<0.05$.

RESULTS

In the present study, the concentrations of trace elements in serum samples of control and thiram groups on day 7 and 14 is given in Table 1 and 2, respectively. Table 1 showed that the concentrations of Cr, Mn, Fe, Co, Ni, Mo, Cd and Pb in thiram group were significantly increased compared with the control groups, whereas the concentration of Cu in thiram group was significantly decreased. However, the concentrations of Cr, Mn, Fe, Ni and Pb in thiram groups on day14 were significantly lower than that of control group ($P<0.05$), and the concentration of Mo was significantly higher as shown in Table 2. The concentrations of Co, Cu and Cd on day 14 in two groups were statistically non-significant.

Table 1: Concentrations of trace elements (mean±SE) in blood serum of control and thiram groups on day 7

Minerals (µg/L)	Control (n=10)	Thiram (n=10)
Cr	1510.04±113.89^{a}	3798.21±188.96^{b}
Mn	88.73±9.51^{a}	253.54±6.33^{c}
Fe	15130.81±1118.25^{a}	40774.96±1353.28^{d}
Co	7.58±0.39^{a}	20.85±0.73^{b}
Ni	275.74±13.42^{a}	686.04±56.07^{d}
Cu	252.03±18.87^{a}	183.42±7.81^{d}
Mo	111.84±3.35^{a}	286.26±10.28^{d}
Cd	1.13±0.054^{a}	3.24±0.19^{d}
Pb	530.74±19.68^{a}	1118.53±22.21^{c}
Zn	1928.64±92.18	2898.98±96.83
Se	246.53±17.05	173.36±4.41
Ti	1073.19±54.92	810.35±21.19
V	25.19±2.05	28.62±0.65
As	23.62±1.46	31.89±2.89

The significance of difference between groups has been shown in superscripts. $^{ab}P<0.001$, $^{ac}P<0.01$, and $^{ad}P<0.05$.

Table 2: Concentrations of trace elements (mean±SE) in blood serum of control and thiram groups on day 14

Minerals (µg/L)	Control (n=10)	Thiram (n=10)
Cr	3109.12±102.89^{a}	1524.12±24.78^{b}
Mn	126.18±7.85^{a}	80.53±0.52^{d}
Fe	27359.73±260.16^{a}	14593.85±274.47^{d}
Co	15.83±0.77	9.12±0.13
Ni	568.96±39.81^{a}	280.84±10.89^{d}
Cu	293.32±9.21	285.12±0.11
Mo	186.75±6.31^{a}	641.67±15.34^{b}
Cd	2.05±0.05	3.93±0.08
Pb	929.12±12.88^{a}	542.71±5.46^{d}
Zn	2543.15±42.94	1772.16±36.49
Se	140.13±3.14	261.92±5.59
Ti	614.85±14.70	855.02±16.73
V	26.78±1.67	23.93±1.94
As	33.00±0.42	21.66±1.36

The significance of difference between groups has been shown in superscripts. $^{ab}P<0.001$, $^{ac}P<0.01$, and $^{ad}P<0.05$.

Table S1: Composition and nutrient levels of the basal diet

Ingredients	Contents (%)	Nutrient levels	Content
Corn	63.8	Digestive energy, MJ/kg	12.02
Soybean	28.0	Crude protein, %	19.80
Fish powder	2.5	Calcium, %	0.90
$CaHPO_4$	1.4	Phosphate, %	0.47
NaCl	0.3	Lysine, %	1.02
Premix	4.0	Methionine, %	0.34

Note: The premix provided the following per kg of diets: Mn 66mg, Zn 44mg, Cu 9mg, Fe 50mg, I 0.4mg, V_A 7000 IU, V_{D3} 875 IU, V_E 20 IU, V_{K3} 1mg, V_{B1} 2mg, V_{B2} 4.5mg, V_{B6} 2.5mg, V_{B12} 0.6mg.

DISCUSSION

Tibial dyschondroplasiais characterized by accumulated growth plate cartilage into metaphyseal region of the tibia, bone deformity and lameness, which affects fast growing broiler chickens worldwide (Shahzad *et al*., 2013). The occurrence of TD leads to huge economic loss to broiler industry worldwide (Shahzad *et al*., 2014). TD is a major metabolic cartilage disease in young poultry whereas trace elements play an important role on bone metabolism, health, osteogenesis and homeostasis. This is because of narrow concentration range of elements that may decide it beneficial or adverse (Dermience *et al*., 2015). In present study, thiram was used to induce TD in broiler chicks that may have effect on glutathione concentration in the growth plate. Glutathione is very important in cellular homeostasis, pathological changes and cell death (Rana *et al*., 2002).

The results of our research indicated that the concentrations of Cr, Mn, Fe, Ni and Pb were high on day 7 and low on day 14 which were different from the control group ($P<0.05$). Trivalent Cr is an essential element in lipid and carbohydrate metabolism (Martin, 2000). However, previous studies have demonstrated that hexavalent Cr can induce many adverse effects, such as accelerating bone resorption by disturbing the release of TGF-β1, IL-β1, TNF-α and TNF-G cytokines and reducing bone formation (Sansone *et al*., 2013), which may be one of the factors to cause TD. Mn plays an important role in bone growth, while it causes abnormal skeletal development in animals when deficit and it impairs bones growth and development when overload (Aschner and Aschner, 2005), which were consistent with our study in which both higher concentration (253.54± 6.33) and lower concentration (80.53±0.52) of Mn were detected in TD chicken compared with control group. Previous studies showed that the deficiency of Fe element can decrease the bone density, strength, disturb bone homeostasis and so on (Medeiros *et al.,* 2002; Harris *et al.,* 2003; Katsumata *et al.,* 2009) which is also agreement with our results in which concentration of Fe was decreased in TD group significantly on day 14. Several studies have proved that the concentration window of Fe element is so narrow that it has caused some genetic diseases (Zarjou *et al.,* 2010; Yang *et al.,* 2011). In this study, the highest (40774.96±1353.28) and lowest concentrations (14593.85±274.47) of Fe induced the occurrence of TD in chickens. Although, in present study Ni concentration did not coincide with the control group, but contrary to other elements it has no effect on bone's growth in birds. Table 1 showed a higher level of Pb (1118.53±22.21) than control group, that can cause osteoporosis and central nervous system exposure (Tsaih *et al.,* 2001), which was in line to another toxic element Cd in this study (3.24±0.19) on day 7. Toxic effects of Cd in bones are well known including increase in osteomalacia, risk of fracture, osteoporosis, disturb osteogenesis, bone homeostasis, calcium metabolism and calciotropic hormones, while decrease in osteoblast viability and alkaline phosphatase activity when the concentration of Cd is too high just like that in the present experiment (3.24±0.19) in Table 1 (Zhu *et al.,* 2004; Brzóska and Moniuszko-Jakoniuk, 2005; Schutte *et al.,* 2008; Yokota and Tonami, 2008; Tahir *et al*., 2017). Conversely, calcium deficiency was also reported that can induce cadmium toxicity (Beattie and Avenell, 1992). We all know that Co is an essential element for vitamin B_{12}. However, several studies investigated that abnormal concentration of Co can affect bone resorption and formation by disturbing bone cell metabolism. Besides, Co also can affect osteoblast size and shape, and decrease alkaline phosphatase levels (Anissian *et al.,* 2002; Fleury *et al.,* 2006; Queally *et al.,* 2009; Sansone *et al.,* 2013). The level of Co in our study was 20.85±0.73, which was significantly higher than the control group, which may be a possible cause of TD. Cu is considered as an important trace element and Table 1 showed the deficiency of Cu (183.42±7.81) in TD chickens. Deficiency of Cu can decrease the bone strength, formation and growth, mineralization and ossification of growth centers inducing hypoplasia, frequent fractures, deformed and brittle bones (Keen *et al.,* 1998; Sarazin *et al.,* 2000). Mo is one of the main compositions of molybdenum enzyme, which promote the process of nitrogen into the food chain (Hille, 2002). However, Mo put adverse effects on animals including growth depression, anemia and reproductive barrier (Cao *et al.,* 2016). Mo can lead to the imbalance of body trace elements and high doses of Mo intake appear to be a result of an induced Cu deficiency (Cao *et al.,* 2016). In the current study, Mo in thiram group was significant higher ($P<0.05$) than control group on day 10, which caused bone damage in TD, as it can cause bone deformities (Cao *et al.,* 2016).

Conclusions: The current study shows that thiram affect the elements in the chick body significantly, which provides consideration to take the effective measures for poultry feed supplement to prevent the occurrence of TD in chickens.

Acknowledgments: This study was supported by the Wenzhou City Public Welfare Science and Technology Plan Projects (N20140041) and Startup Project of Doctor scientific research of Wenzhou Vocational College of Science and Technology in 2016 (201604) and College Students' Science and Technology Innovation Project of Zhejiang Province in 2017 (2017R458001).

Authors contribution: KL designed the study. KL, HQL, SJW, YB, HZ, KM, MM and JXW performed the experiments and KL, HQL and JXW analyzed the data. K L and KM wrote the manuscript. All authors read and approved the final manuscript.

REFERENCES

Anissian L, Stark A, Dahlstrand H, *et al.,* 2002. Cobaltions influence proliferation and function of human osteoblast-like cells. Acta Orthop 73:369-74.

Aschner JL and Aschner M, 2005. Nutritional aspects of manganese homeostasis. Mol Aspects Med 26:353-62.

Barany E, Bergdahl IA, Bratteby LE, *et al.,* 2002. Relationships between trace element concentrations in human blood and serum. Toxicol Lett 134:177-84.

Beattie JH and Avenell A, 1992. Trace Element Nutrition and Bone Metabolism. ROYAUME-UNI: Cambridge University Press Cambridge.

Beede DK, 2006. Evaluation of water quality and nutrition for dairy cattle. High Plains Dairy Conference Amarillo, Texas.

Brzóska MM and Moniuszko-Jakoniuk J, 2005. Disorders in bone metabolism of female rats chronically exposed to cadmium. Toxicol Appl Pharmacol 202:68-83.

Cao HB, Gao FY, Xia B, *et al.,* 2016. The co-induced effects of molybdenum and cadmium on the mRNA expression of inflammatory cytokines and trace element contents in duck kidneys. Ecotox Environ Safe 133:157-63.

Dermience M, Lognay G, Mathieu FO, *et al.*, 2015. Effects of thirty elements on bone metabolism. J Trace Elem Med Bio 32:86-106.

Fleury C, Petit A, Mwale F, *et al.,* 2006. Effect of cobalt and chromiumions on human MG-63 osteoblasts in vitro: morphology, cytotoxicity and oxidative stress. Biomaterials 27:3351-60.

Harris MM, Houtkooper LB, Stanford VA, *et al.,* 2003. Dietary iron is associated with bonemineral density in healthy postmenopausal women. J Nutr133:3598-602.

Hille R, 2002. Molybdenum and tungsten in biology. J Trends Biochem Sci 27:360-7.

Hostetler CE, Kincaid RL and Mirando MA, 2003 The role of essential trace elements in embryonic and fetal development in livestock. Vet J 166:125-39.

Katsumata SI, Katsumata-Tsuboi R, Uehara M, *et al.*, 2009. Severe iron deficiency decreases both bone formation and bone resorption in rats. J Nutr 139:238-43.

Keen CL, Uriu-Hare JY, Hawk SN, *et al.,* 1998. Effect of copper deficiency on prenatal development and pregnancy outcome. Am J Clin Nutr 67:1003S-11S.

Leach RM and Monsonego-Ornan E, 2007. *Tibial dyschondroplasia* 40 years later. Poult Sci 86:2053-8.

Martin A, 2000. Apports Nutritionnels Conseilles Pour La Population Francaise, Third ed, Tec & Doc Editions, Paris, 2000.

Medeiros DM, Plattner A, Jennings D, *et al.*, 2002. Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. J Nutr 132:3135-41.

Queally JM, Devitt BM, Butler JS, *et al.,* 2009. Cobalt induce chemokine secretion in primary human osteoblasts. J Orthop Res 27:855-64.

Rasaputra KS, Liyanage R, Lay JQ, *et al.*, 2013. Effect of thiram on avian growth plate chondrocytes in culture. J Toxicol Sci 38:93-101.

Rana SV, Allen T and Singh R, 2002. Inevitable glutathione. Indian J Exp Bio 40:706-16.

Sansone V, Pagani D and Melato M, 2013. The effects on bone cells of metal ions released from orthopaedic implants, a review. Clin Cases Mineral Bone Metab 10:34-40.

Sarazin M, Alexandre C and Thomas T, 2000. Influence of trace element, protein, lipid carbohydrate, and vitamin intakes on bone metabolism. Rev Rhum 67:486-97.

Schutte R, Nawrot TS, Richart T, *et al.,* 2008. Bone resorption and environmental exposure to cadmium in women: a population study. Environ Health Perspect 116.

Shahzad M, Liu JY, Gao JF, *et al.,* 2014. Hsp-90 Inhibitor geldanamycin aAttenuates liver oxidative stress and toxicity in thiram-induced *Tibial Dyschondroplasia*. Pak Vet J 34:545-7.

Tahir MW, Saleemi MK, Khan A, *et al*., 2017. Hematobiochemical effects of cadmium intoxication in male Japanese quail (*Coturnix japonica*) and its amelioration with silymarin and milk thistle. Toxin Rev 36:187-93.

Tsaih SW, Korrick S, Schwartz J, *et al.,* 2001. Influence of bone resorption on the mobilization of lead from bone among middle-aged and elderly men: The Normative Aging Study. Environ Health Perspect 109:995-9.

Yang Q, Jian J, Abramson SB *et al.*, 2011. Inhibitory effects of iron on bone morphogenetic protein 2–induced osteoblastogenesis. J Bone Miner Res 26:1188-96.

Yokota H and Tonami H, 2008. Experimental studies on the bone metabolism of male rats chronically exposed to cadmium intoxication using dual-energy X-ray absorptiometry. Toxicol Ind Health 24:161-70.

Zarjou A, Jeney V, Arosio P, *et al.,* 2010. Ferritin ferroxidase activity: a potent inhibitor of osteogenesis. J Bone Miner Res, 25:164-72.

Zhu G, Wang H, Shi Y, *et al.,* 2004. Environmental cadmium exposure and forearm bone density, Biometals 17:499-503.