İstanbul Üniv. Vet. Fak. Derg.
28 (1), 105 - 112, 2002

J. Fac. Vet. Med. Univ. Istanbul
28 (1), 105 - 112, 2002

THE EFFECT OF DIETARY SUPPLEMENTATION OF ZINC AT TOXIC LEVEL ON THE PERFORMANCE AND THE ZINC CONCENTRATIONS OF SOME TISSUES IN BROILERS

Derviş ÖZÇELİK* Şefik DURSUN* Recep KAHRAMAN**
Neşe KOCABAĞLI** Müjdat ALP** Gülsüm E. ÖZTEKİN***

Broyler yemine toksik düzeyde katılan çinko sülfatın performansa ve bazı doku çinko konsantrasyonlarına etkisi

Özet: Araştırmada, yumurtadan çıkmadan itibaren dört hafta süreyle diyetlerine toksik düzeyde çinko katılan broyler civcivlerinin performansı ile kan, karaciğer, böbrek, göğüs eti ve kemikte çinko birikimine etkileri incelenmiştir.

Denemede 60 adet günlük et tipi Avian hibrit civcivler her birinde 30 adet civciv bulunan biri kontrol, diğeri çinko sülfat (2500 mg/kg yem) ilaveli yemle beslenen grup olmak üzere 2 gruba ayrılarak civciv bölmelerine yerleştirilmiştir.

Denemenin sonunda, kontrol grubundaki broylerlerin canlı ağırlığının yemlerine çinko sülfat katılan broyler grubundan %282.2 daha yüksek olması istatistiksel yönden önemli bulunmuştur ($p<0.001$). Kontrol grubundaki broylerlerin yemden yararlanması yemlerine çinko sülfat katılan broyler grubundan %243.5 daha iyi olmuştur.

Kontrol ve diyetine toksik düzeyde çinko sülfat katılan broyler gruplarının serum, karaciğer, böbrek, göğüs eti ve kemikteki düzeyleri incelendiğinde, çinko grubundaki broylerlerde doku çinko düzeyleri kontrol grubuna göre sırasıyla, %327.9; %1004.4; %390.2; %68.0 ve %615.6 daha fazla bulunmuştur ($p<0.001$).

Sonuç olarak, diyete toksik düzeyde katılan çinkonun performansı olumsuz etkilemesinin yanında, en çok karaciğer ve böbrekte biriktiği saptanmıştır.

Anahtar Kelimeler: Broyler, çinko, toksisite, performans, doku konsantrasyonu.

Summary: The aim of this study was to investigate the accumulation of the toxic doses of zinc in several tissues taken with diet and their effects on performance of broiler chicks. For this purpose, 60 one day-

* University of Istanbul, Faculty of Cerrahpaşa Medicine, Department of Biophysics, 34303 Cerrahpaşa / Istanbul - Turkey.

** University of Istanbul, Veterinary Faculty, Department of Animal Nutrition and Nutritional Diseases, 34 851, Avcılar / Istanbul - Turkey.

*** University of Istanbul, Faculty of Cerrahpaşa Medicine, Department of Gastroenteroloji, 34303 Cerrahpaşa / Istanbul - Turkey.

old chicks were used and they were evenly divided into two groups consisting controls and experimental broilers. Chicks in group were fed with normal ration, while the experimental group were fed with diets containing 2500 ppm zinc.

At the end of the fourth week, the chicks were slaughtered. The average live weight of the control chicks were 282.2 % higher than the experimental group and the difference was statistically significant ($p<0.001$). In addition, feed conversion rate of the control group was 243.5 % higher than the experimental group.

When the mineral substance levels in serum, liver, kidney, muscle and bone of the control group and the experimental group analysed, it was found that the different was statistically significant ($p<0.001$). The zinc concentrations in the tissue of experimental group were 327.9 %, 1004.4 %, 390.2 % 68.0 % and 615.6 % respectively and higher than the control group.

The results of this study indicates that the intake of toxic doses of zinc might decrease broiler performance and causes accumulation in tissues with higher content ration in the liver and kidney.

Key Words: Broiler, zinc, toxicity, performance, tissue concentrations.

Introduction

Zinc is one of the essential element for the normal functioning and development of the broilers. It is proposed that zinc is the most important mineral substance among the other essential elements after iron (8, 14).

Only 15-30% of zinc in a diet can be absorbed. The absorption of zinc from the small intestine, mainly occurs in duodenum and at the proximal part of jejunum. There is absolutely no absorption from the large intestine (4, 14).

The important portion of the zinc, that comes from diet passes into blood, and binds to proteins. In blood plasma, zinc is mostly carried by binding to albumin (60-70%) and α-2 macroglobulin (30-40%). A small amount is carried by transferrin and free amino acids (9). As the level of zinc in the diet increases, plasma, liver, kidney, bone and muscle zinc levels also increases. In addition, bone is the most sensitive tissue for zinc accumulation. Liver and kidney comes after bone (9, 10, 12). Prolonged intake of zinc more than requirement, changes the metabolism of other essential elements (15). In fact, zinc is a non-toxic element. But the soluble salts of zinc have major toxicity. Soluble zinc salts may cause on acute poisoning, especially in the presence of acids and acidic substances (1, 8).

It is proposed that the presence of zinc, 50-100 times of the normal level, in animal rations leads to decrease in the body weight gain due to a reduction in feed consumption (1, 15). It is also stated that the addition of 4000 ppm zinc to diet decreases the live weight increase, and also feed consumption rate and feed conversion ratio (15).

In this study, toxic levels of zinc were added to chicks' diets for four weeks, and the performance of day-old chicks and the effects of zinc accumulation in serum, liver, kidney, muscle and bone tissues were investigated.

Material and Methods

Sixty day-old Avian hybrid chicks were used in our experiment and were divided evenly into control and experimental group. The chicks in the control group were fed with on ordinary broiler diet. The experimental group were fed with diets which contained 2500 ppm zinc. The diet used for control chicks was formulated by linear programming to meet the nutrient needs suggested by the National Research Council (7). The composition of the basal diet is shown in Table 1. The toxic dose of zinc was added to the basal diet of the experimental group as zinc sulphate ($ZnSO_4 \cdot 7H_2O$).

Tablo 1. Composition and nutrients content of the broiler diet.

Ingredients and composition	Diet, 0 to 28 days %
Yellow corn	40.00
Wheat fine	21.00
Soybean meal (44%)	15.00
Sunflower meal (32%)	5.70
Fish meal (62%)	4.20
Poultry by-product (56% CP)	5.00
Vegetable oil	5.00
Limestone	1.30
Dicalcium phosphate	1.20
Vitamin & mineral premixes[1]	0.40
Salt	0.25
Lysine	0.08
DL-Methionine	0.17
Coccidiosit	0.10
Antioxidant	0.10
Calculated analysis	
AME (kcal/kg)	2803
Lysine, %	1.20
Methionine + Cystine, %	0.85
Analysed values	
Crude protein, %	18.08
Calcium, %	1.42
Total phosphorus, %	0.56

1 Premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 1,500 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; vitamin B_{12}, 0.007 mg; thiamine, 0.4 mg; riboflavin, 6 mg; folic acid, 1 mg; biotin, 0.15 mg; pantothenic acid, 12 mg; niacin, 35 mg; pyridoxine, 4 mg; choline, 1,000 mg; iodised salt, 2 g; manganese, 60 mg; copper, 5 mg; zinc, 50 mg; and selenium, 0.1 mg.

The chicks were randomly assigned to two floor pens (2 m^2) in a broiler test house. The pens contained litter composed of new wood shavings. Each pen was equipped with one hanging feeder and one automatic water fount. During the first 2 weeks, supplemental heat was provided, and the birds were confined to the heated area. Supplemental feeder flats and waterers were used during the first 7 days. Both feed and water were given ad *libitum* and, florescent lighting was provided continuously.

The trial ended in 4 weeks. In order to determine the weight performances of chicks in control and experimental groups, weekly live weight measurements were recorded individually.

On the twenty-eighth day of the trial, chicks were slaughtered and blood samples were collected into plastic tubes. In order to measure zinc concentration, serum was separated from blood samples and serum samples of all animals were kept at -20°C until required (3, 6, 13). The liver, kidney, muscle and bone (right tibia) samples of chicks were taken in order to determine tissue zinc concentrations. For element determinations, atomic absorption ready-made standard solutions (Titrosol-Merck, Germany) containing 1.000 ± 0.002 mg pure element (Zn) completed to one litre with deionized water and stock solutions (1000 µg/ml) were prepared. For zinc measurement, working standards of 0.5, 1, 2 µg/ml were obtained from deionized water and stock solutions.

In the atomic absorption spectrophotometer, HLC (Hollow Cathode Lamp) which gives light at a special wavelength and slits space suitable for element (Zn), air-acetylene gaseous mixture and BCG (Background) modes were chosen. For the atomic absorption spectrophotometer which was ready for doing measurements in this manner, calibration graphics were dawn for the element whose measurement was to be made with the help of the standard solutions. Zinc level in serum was measured directly by flame atomic absorption spectrophotometer (Shimadzu 680 AA). Zinc concentrations in tissues were determined by flame atomic absorption spectrophotometer after samples had been wet digested ashing in 65% HNO_3 at approximately 100-120°C for 2h and properly diluted with demineralized water.

Basal diet was chemically analysed for crude protein, Ca and P according to the AOAC (2) methods. Data for the feed conversion ratio could not be statistically analysed because of the group feeding. The data of body weight gain and zinc concentrations of some tissues were analysed by Student's t-test (11). The difference was considered to be significant if the P value was ≤ 0.05.

Results

The initial and weekly live weight medians when toxic levels of zinc sulphate are added into chick feed, standard errors, the importance controls between the groups according to the "t" test are presented in Table 2. In the statistical analyses, the differences between the groups were found to be statistically significant ($p<0.001$) in all weeks except the beginning of experiment.

Tablo 2. Average body weights of control and zinc supplemented diet.

Groups	Age, week														
	0			1			2			3			4		
	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$
Control	30	40.53	0.49	30	148.55	2.08	29	331.67	6.01	28	611.79	12.63	23	850.44	20.31
Zinc sulphate	30	40.45	0.61	30	82.92 ***	2.10	19	132.66***	6.86	13	152.31***	11.35	8	222.50***	22.42

*** (P<0.001)

At the end of the fourth week, the chicks were slaughtered. The average live weight of the chicks in the control group were 282.2% and was higher than the chicks in the experimental group and it was found statistically significant (p<0.001).

At the end of the 4th week of trial period, feed conversion rate of the control group was 243.5% and was higher than the experimental group (Table 3).

Tablo 3. Effect of diets on feed conversion ratio of broilers.

Groups	Age, week			
	0-1	0-2	0-3	0-4
Control	1.37	1.48	1.59	1.91
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	1.47	2.46	3.97	6.56

Table 4 shows, the mineral concentrations in blood, liver, kidney, muscle and bone tissues of the experimental chicks and the statistical analysis of the data. When the mineral substance levels in serum, liver, kidney, muscle and bone of the control group and the experimental group were compared, the tissue mineral concentrations in the experimental group were 327.9%, 1004.4%, 390.2%, 68.0% and 615.6% respectively and were higher than in the control group (Table 4). The difference amongst groups was statistically significant (p<0.001).

Tablo 4. The mean and standard deviations of the measured zinc concentrations in the mentioned tissues of the control and experimental animals and the percentage change values of tissue zinc concentrations of the experimental groups.

Groups	n	Serum µg/ml	Liver µg/g	Kidney µg/g	Muscle µg/g	Bone µg/g
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Control	23	161.77 ± 77.00	24.65 ± 4.22	29.79 ± 9.05	6.57 ± 0.78	210.87 ± 37.57
Zinc sulphate	8	692.26 *** ± 367.86	272.23 *** ± 80.74	146.04 *** ± 77.00	11.04 *** ± 3.10	1509.07 *** ± 594.4
Percentage Change, %		**327.9**	**1004.4**	**390.2**	**68.0**	**615.6**

*** (P<0.001)

Discussion

In this study, the weekly live weight gain of the chicks whose diets contained toxic level of zinc (2500 ppm) was 282.2% and was lower than the control group (Table

2). According to the NRC (National Research Council) (7) proposals, it is proposed that the presence of 40 ppm of zinc which is an essential element in chick diet is enough. It is stated that the addition of zinc at a toxic level of 4000 ppm into the diet reduces live weight gain, feed consumption and feed conversion ratio (15). Otherwise, feed conversion rate of the control group was 243.5% better than the experimental group (Table 3). The results of this study indicates that the addition of high amounts of zinc into chick diet effects the live weight gain in a negative way, by both reducing the feed conversion ratio and by the interaction of zinc with other mineral substances.

The finding, which were found statistically significant for ($p<0.001$) high levels of mineral substance in serum, liver, kidney, muscle and bone tissues of chicks whose diets contained toxic levels of zinc sulphate (Table 4), support the hypothesis that liver, muscle and blood levels of zinc in chicks increase parallel with the zinc concentrations in the diet (5, 10, 12, 15).

In this study, the level of zinc in bone and liver was higher than in the other tissues and is similar to those reported by others (10, 12, 15). These investigators emphasized that liver, muscle and blood zinc levels also increase depending on the zinc concentration of the diet. The percentage differences of zinc concentrations in tissues of experimental group chicks were seen mostly in the liver and bone. In the same way, it is stated that when excess level of zinc is taken by diet, zinc mostly accumulates in bone tissue followed by the liver and kidney.

The intake of essential elements consistently at a toxic level deteriorates the metabolism of the tissues and organs in which it accumulates. Especially in animals that fed by zinc-rich diets, developmental derangement, decrease in performance and deaths are encountered. In conclusion, the intake of trace elements of toxic levels might cause accumulation in higher concentrations in same tissues. Therefore, essential mineral substances should not be included in the chicks diet more than its requirement. Otherwise, developmental deterioration is inevitable and most importantly, the consumption of such contaminated food may lead to various health problems in people.

References

1. **Ammerman, B.C., Baker, H.D. and Lewis, A.J. (1995):** Bioavailability of Nutrients for Animals Amino Acids, Minerals and Vitamins. Academic Press. Inc., New York, 367-375.
2. **AOAC (1984):** Official Methods of Analysis. 14th ed., Association of Official Agricultural Chemist, Washington, D.C.
3. **Brown A. and Taylor, A. (1985):** Applications of a slotted quartz tube and flame atomic-absorption spectrometry to the analysis of biological samples. Analyst., June, (110) 579-582.
4. **Burch, R.E., Kahn, H.K.J. and Sullivan, J.F. (1975):** Newer aspects of the roles of Zn, Mn and Cu in human nutrition. Clin. Chem., 21: 501-520.

5. **Henry, P.R., Ammerman, C.B. and Miles, R.D. (1987):** Effect of dietary zinc on tissue mineral concentration as a measure of zinc bioavailability in chicks. Nutrition reports International, 35 (1) 15-23.

6. **Nancy, W. (1982):** Serum versus plasma for trace metal analysis. In: Trace Element Metabolism in Man and Animals, J. M. Gawthorne ed., New York.

7. **National Research Council (1994):** Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

8. **Prasad, A.S. (1985):** Clinical endocrinological and biochemical effects of zinc deficiency. Clin. Endocrinol. Metabolism, 14: 567.

9. **Prasad, A.S. (1978):** Trace Elements and Iron Human Metabolism. In John Wiley and Sons, Great Britain, 289-303.

10. **Shan, A.S. (1993):** Effects of fiber and various zinc compounds on performance, blood biochemical parameters and zinc concentrations in tissues of chicks. Acta Veterinaria et Zootecnica Snica, 24 (1) 29-35.

11. **Snedecor, G.W. and Cochran, W.G. (1980):** Statistical Methods. 7th ed., The Iowa State Univ. Press, Ames, Iowa.

12. **Stahl, J.L., Janet, L. and Greger, C.M.E. (1989):** Zinc, copper and iron utilisation by chicks fed various concentrations of zinc. British Poultry Sci., 30: 123-134.

13. **Taylor, A. and Brown, A. (1983):** Simple and rapid procedure for the determination of lead whole blood by use of a slotted tube and discrete nebulisation flame atomic-absorption spectrometry, Analyst., September 7, 1159-1161.

14. **Tietz, N. (1986):** Textbook of Clinical Chemistry. W.B. Saunders Company, 965-985.

15. **Watkins, K.L. and Southern, L.L. (1993):** Effect of dietary sodium zeolit A on zinc utilisation by chicks. Poultry Sci., 72: 296 - 305.