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The Accumulation Levels in some Tissues and the Effect on Lipid Fractions in Liver of Dietary Aluminum in Broilers

Ayşen ZOHOURİ, Süleyman K. TEKELİ, Ahmet MENGİ
Department of Biochemistry, Faculty of Veterinary Medicine, University of Istanbul-TURKEY
Esma TÜTEM, Mehmet HÜGÜL
Department of Chemistry, Engineering Faculty, University of Istanbul, Istanbul-TURKEY

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Abstract: In this study, the accumulation levels in serum, muscle and bone and the effect on lipid fractions in liver of dietary aluminum in broilers were examined.

Day-old 30 broiler chicks were separated into two equal groups. Experimental group was fed with diet added 2 g/kg aluminum sulfate. Feed and water were given ad libitum. At the end of 40-day, the blood samples were taken from chickens and the sera were separated. In same day, all chickens were killed, and liver, bone and muscle samples were removed. Al determinations in serum, bone, muscle, feed and drinking water were done. Cholesterol, triglyceride and total lipid analyses in liver were done.

Serum Al levels were not found different between control and experimental groups. The Al levels in bone and muscle were significantly found different among these groups ($p<0.001$). Dietary aluminum did not change cholesterol and total lipid content of liver, whereas it significantly reduced triglyceride content ($p<0.01$).

Key Words: Aluminum, broiler, accumulation levels, lipid fractions of liver.

Broilerlerde Yeme Katılan Alüminyumun Bazı Dokulardaki Birikim Düzeyleri ve Karaciğerdeki Lipid Fraksiyonları Üzerine Etkisi*

Özet: Bu çalışmada broilerlerde yeme katılan alüminyumun serum, kas ve kemikte birikim düzeyleri ile karaciğerin lipid fraksiyonları üzerindeki etkisi incelenmiştir.

30 adet 1 günlük broiler civciv 2 eşit gruba ayrılmıştır. Deneme grubu 40 gün süresince 2 g/kg alüminyum sülfat ilave edilen yemle beslenmiştir. Yem ve su ad libitum verilmiştir. 40 günün sonunda tavuklardan kan alınarak serumları ayrılmıştır. Aynı gün tüm tavuklar kesilerek karaciğer, kemik ve kas numuneleri alınmıştır. Serum, kemik, kas, yem ve su numunelerinde Al tayinleri yapılmıştır. Karaciğerde kolesterol, trigliserid ve total lipid analizleri yapılmıştır.

Serum Al düzeyleri kontrol ve deneme grubu arasında farklı bulunmamıştır. Kemik ve kastaki Al düzeyleri ise her iki grup arasında $p<0.001$ düzeyinde farklı bulunmuştur. Yeme eklenen alüminyum karaciğerin kolesterol ve total lipid içeriğini değiştirmediği halde trigliserid içeriğini $p<0.01$ düzeyinde azaltmıştır.

Anahtar Sözcükler: Alüminyum, broiler, birikim düzeyleri, karaciğerin lipid fraksiyonları.

Introduction

Aluminum is the most abundant metal in the lithosphere, but its levels in natural waters, plants, and animals are low. During food preparation (Al ware, direct food additives) and transportation (Al packaking, containers) the Al concentrations may increase to levels which are not regarded as a health hazard. Industrial activities and automobile particulate emissions contribute to atmospheric pollution (1,2).

Aluminum enters the organism via gastrointestinal tract (food, beverages, water) and lungs (airborne dust). Total daily intake is estimated to be approximately 80 mg. Gastrointestinal absorption is low and interferes with phosphate and iron absorption (3,4,5). In blood plasma, Al is bound to nondialyzable components. It is stored in lung and hilar lymph nodes from inhaled air. Increased doses of Al (in food or parenterally) give rise to its concentrations mainly in the brain, liver, and blood (1,3).

Aluminum specifically activates the myocardial succinate oxidase system (nicotinamide adenine

dinucleotid requiring, cytochrome C containing). Fibrotic lung reaction and alteration of phosphate metabolism are the critical toxic effects of Al. Increased Al ingestion reduces the intestinal absorption of P, due to formation of insoluble Al phosphate complexes (1,6,7,8,9). As a result, P and Ca metabolism (impaired growth, rachitic bone changes, increased Al level in bone) and the parathyroid hormone level in serum (correlated to serum Al level) are affected (2,10,11). At a cellular level, high Al concentrations suppress the phosphorylation, resulting in reduction of blood adenosine triphosphate / adenosine diphosphate (ATP/ADP) ratio and the level of ATP. Excess of Al in the cells alters phosphate transferring enzyme systems involving ATP and Mg, with parallel disturbances in carbohydrate metabolism. Al stimulates the enzymatic decarboxylation of pyruvic acid, inhibits hexokinase as well as the conversion of isocitric acid to α-ketoglutarate (nicotinamide adenine dinucleotide phosphate dependent). Other biochemical effects of Al excess are coagulopathy (prolonged prothrombin time) and porphyrinuria (1).

In humans, toxic concentrations of Al are supposed to have ethiological significance in primary degenerative dementia (Alzheimer's disease or senile dementia), in dialysis dementia, and in dialysis-related osteomalacia. The high Al-levels found in brain tissue and brain regions of patients who died from primary degenerative dementia bear out the ethiological role of Al in this disease. Aluminum toxicity is involved in the dialysis encephalopathy syndrome as well as in osteomalacia in encephalopathic cases. Al absorption and accumulation in blood and tissues (especially in brain gray matter) occurs in patients with chronic renal failure on dialysis (1,3,12).

Limited information is available concerning the nutrition and metabolism of aluminum in avian species. Deobald and Elvehjem (13) demonstrated that 0.22-0.44 % dietary aluminum, supplied as aluminum sulfate, depressed growth rate, bone mineralization and serum phosphorus levels in young chicks. Wisser et al. (6) reported that added aluminum at 0.30 % severely depressed body weight and feed efficiency and significantly perturbed bone mineralization and plasma phosphorus levels but did not produce rachitic lesions. Rosa et al. (14) found increased bone Ca in response to increased dietary P which was lowered when Al was added to the diet. Nyholm (15) reported on an adverse effect of Al on apatite formation in bone marrow and poor egg shell quality of wild passerine birds. High levels of dietary Al have been shown to depress feed intake and to reduce the availability of dietary P. The addition of 0.3 % Al to a diet containing 0.35 % available P depressed feed intake and body weight of SCWL hens (16).

The objectives of the present research were to examine whether or not diet contamined with high aluminum levels through various sources leads to aluminum deposits in serum, muscle and bone tissues of broilers and to determine if high dietary aluminum results in fatty liver degeneration.

Materials and Methods

The experiment was conducted utilizing day-old 30 broiler chicks. They were randomly divided into two equal groups as control and experimental groups. Feed and water were provided for ad libitum intake throughout the 40-day experimental period.

Dietary aluminum supplementations of experimental group were made with aluminum sulfate ($Al_2(SO_4)_3.18H_2O$). The sulfate content of the aluminum sulfate was not considered to be a complicating factor in this experiment. For control group, sodium sulfate was fed to provide levels of the anion equal to those supplied by aluminum sulfate in diet of experimental group containing 2 g/kg aluminum.

At the end of the 40-day experimental period, the blood samples were taken from each chicken by jugular venipuncture. They were centrifugated and, serum samples were stored frozen (- 20℃) in polyethylene vials. The chickens were killed by cervical dislocation. The left tibia and surrounding muscles were removed. Livers were removed for the determination of lipid fractions.

To minimize aluminum contamination from glassware, all laboratory equipment used was made of polyethylene plastics. All equipment was washed in a plastic dish with deionized water and dried prior to use. The deionized water was stored in a large polyethylene bottle.

Serum samples were extracted with trichloracetic acid (7). One-gram muscle samples were dried at 75℃ for 24 h, and ashed at 600℃ for 6 h (17). Tibiae were cleaned and defatted in an ethanol / benzene extraction solution. The dry bones were then ashed at 600℃ overnight. Aluminum determination was performed on 200 mg bone ash samples. Ingredients were weighed and mixed using plastic equipment. One-gram samples were weighed and, ashed at 600℃

overnight. Ashed tissues were digested in nitric acid, and diluted in deionized water for Al determination (6). Al levels of all samples (including drinking water) were determined spectrophotometrically (18).

Liver samples were homogenized with 0.25 M saccarose. Obtained homogenates were centrifugated and, supernatants were separated. Total lipid, cholesterol and triglyceride levels were measured spectrophotometrically (19).

Differences among treatment means were determined by using the test of least significant difference when a significant value of F was obtained in the analysis of variance (20).

Results

In this study, the aluminum levels in basal diet and drinking water were found 14.83 and 0.07 µg/g, respectively.

Serum, bone and muscle aluminum data are presented in Table 1. Serum Al levels were not significantly different between control and experimental groups. Bone and muscle Al concentrations were significantly higher in hens receiving 0.2 % aluminum than control hens.

Table 1. The levels of aluminum determined in serum, bone and muscle samples (n=8).

Samples	Control group (µg/g)		Experimental group (µg/g)	
	x	SEM	x	SEM
Serum	1.34	0.089	1.47	0.036
Bone	11.59	0.496	19.21***	0.740
Muscle	3.48	0.256	6.26***	0.219

*** p<0.001
SEM Standard error of mean

Table 2. The effect of dietary aluminum on lipid fractions of liver (n=15).

Parameters (µg/100)	Control group x	SEM	Experimental group x	SEM
Cholesterol	108.9	8.74	95.1	11.91
Triglyceride	392.9	11.18	338.0**	11.76
Total lipid	978.2	1.69	968.3	4.48

** p<0.01
SEM Standard error of mean

Cholesterol, triglyceride and total lipid levels of liver were presented in Table 2. Although triglyceride concentration in experimental group was significantly different from that of control group, cholesterol and total lipid levels showed no significant changes between experimental and control groups.

Discussion

In this study, the aluminum levels in basal diet was 14.83 µg/g. Wisser et al. (6) reported that commercial feeds for young chicks were found to range between 0.003 and 0.01 % aluminum. They also reported that of the individual feed ingredients, soybean meal was the major source of aluminum.

Sooncharernying and Edwards (21) found that supplemental Al as low as 250 mg/kg had an adverse effect on feed efficiency and 500 mg/kg decreased body weight and bone ash. Storer and Nelson (22) found that levels of 0.1 to 0.4 % Al as aluminum sulfate decreased growth, bone ash, and feed efficiency in SCWL chicks. In the studies of Deobald and Elvehjem (13) 0.22 to 0.44 % Al as the sulfate depressed growth of day-old SCWL chicks. Hussein (23) showed that the decreased growth of broiler chicks fed Al was mostly due to reduced feed intake, but was also due to a metabolic effect of Al per se. However, Berlyne et al. (24) and Lipstein and Hurwitz (7) reported that the primary effect of Al toxicity is to deplete P, thereby indirectly affecting feed intake and body weight.

Aluminum can bind with P, thereby reducing the availability of P (25). Reducing P can increase bone resorption (26). Cox et al. (27) observed a 15 % reduction in plasma Pi and about a 70 % depression in bone ash due to feeding Al to guinea pigs. Feeding high levels of Al to rats resulted in rickets or decreased plasma P or both (28). Peo et al. (29) noted that 372 ppm Al as aluminum sulfate reduced bone breaking strength in pigs. Wisser et al. (6) reported that plasma calcium showed an inconsistent response to dietary aluminum and, hens fed 0.15 % added aluminum had significantly higher plasma calcium values than control birds. They also reported that no differences existed in plasma phosphorus levels, and bone analysis showed no differences in percentage ash or in calcium and phosphorus concentrations. According to same investigators, these results suggest that aluminum interferes with other

systems in addition to phosphorus metabolism.

In this study, Al levels of serum and bone in treated group were 1.47 and 19.21 μg/g, respectively. Wisser et al. (6) reported that bone ash was significantly reduced and bone aluminum was significantly increased by added aluminum, both in a dose-dependent manner. They found that bone Al levels in laying hens with 0.00, 0.15 and 0.30 % added Al were 8.3, 11.7 and 19.1 μg/kg, respectively and, they were 1.49, 6.21 and 9.73 μg/kg in chicks. Hussein et al. (30) found that plasma Al was not affected by dietary Al. This finding is parallel to our results.

Szilagyi et al. (31) reported that the treated groups showed significantly elevated alkaline phosphatase activities, as well as increased cholesterol concentrations and decreased triacylglycerol concentrations in serum, and these changes were dose-dependent. They also supposed that high levels of alkaline phosphatase are due to increased osteoblastic activity, provoked by the disturbance of bone formation, caused in turn by aluminum.

The results of this study indicate that dietary Al accumulated in bone and muscle tissues but not in serum and reduced the triglyceride content of liver.

References

1. Tsalev, D.L., Zaprianov, Z.K.: Atomic absorption spectrometry in occupational and environmental health practice. Volume 1, CRC Press, Inc. Boca Raton, Florida, 1985.
2. Hussein, A.S., Cantor, A.H., Johnson, T.H.: Effect of dietary aluminum on calcium and phosphorus metabolism and performance of laying hens. Poultry Sci., 1989, 68:706-714.
3. Farrar, G., Blair, J.A.: A proposed model to account for the accumulation of aluminium in Alzheimer brain. In: Proceedings of the Third International Congress on Trace Elements in Health and Disease at Adana (Turkey) March 31-April 8, 1989.
4. Elliot, M.A., Edwards, H.M.,Jr.: Some effects of dietary aluminum and silicon on broiler chickens. Poultry Sci., 1991, 70: 1390-1402.
5. Spencer, H., Kramer, L., Norris, C., Wiatrowski, E. : Effect of aluminum hydroxide on plasma fluoride and fluoride excretion during a high fluoride intake in man. Toxicol. Appl. Pharmacol., 1981, 58:140.
6. Wisser, L.A., Heinrichs, B.S., Leach, R.M.: Effect of aluminum on performance and mineral metabolism in young chicks and laying hens. J. Nutr., 1990, 120: 493-498.
7. Lipstein, B., Hurwitz, S.: The effect of aluminum on the phosphorus availability in algae-containing diets. Poultry Sci., 1982, 61: 951-954.
8. Lipstein, B., Hurwitz, S.: The nutritional value of sewage-grown, alum-flocculated micractinium algae in broiler and layer diets. Poultry Sci., 1981, 60:2628-2638.
9. Hussein, A.S., Cantor, A.H., Johnson, T.H.: Use of high levels of dietary aluminum and zinc for inducing pauses in egg production of Japanese quail. Poultry Sci., 1988, 67: 1157-1165.
10. Allen, V.G., Fontenot, J.P.: Influence of aluminum as sulfate, chloride and citrate on magnesium and calcium metabolism in sheep. J. Anim. Sci., 1984, 59,3: 798-804.
11. Mayor, G.H., Keiser, J.A., Ku, P.K.: Aluminum absorption and distribution. Effect of parathyroid hormone. Science, 1977, 197: 1187.
12. Alfrey, A.C.: Aluminum intoxication. N. Engl. J. Med. 310:1113-1114.
13. Deobald, H.J., Elvehjem, C.A.: The effects of feeding high amounts of soluble iron and aluminum salts. Am. J. Physiol., 1935, 111: 118-123.
14. Rosa, I.V., Henry, P.R., Ammerman, C.B.: Interrelationship of dietary phosphorus, aluminum and iron on performance and tissue mineral composition in lambs. J. Anim. Sci., 1982, 53: 1231.
15. Nyholm, N.E.I.: Evidence of involvement of aluminum in causation of defective formation of eggshells and of impaired breeding in wild passerine birds.Environ. Res., 1981, 26: 363-371.
16. Hussein, A.S., Cantor, A.H., Johnson, T.H.: Comparison of the use of dietary aluminum with the use of feed restriction for force-molting laying hens. Poultry Sci., 1989, 68: 891-896.
17. Roland, D.A.,Sr., Rabon, H.W.,Jr., Rao, K.S., Smith, R.C., Miller, J.W., Barnes, D.G., Laurent, S.M.: Evidence for absorption of silicon and aluminum by hens fed Sodium Zeolite A. Poultry Sci., 1993, 72: 447-455.
18. Sandell, E.B.: Colorimetric determination of trace of metals. 4. Edition, Interscience publicians Ltd. London, 1959.
19. Teco Diagnostics. Placentia, CA 92670 USA, 1995.
20. Snedecor, G.W., Cochran, W.G.: Statistical methods, 7. Edith. The Iowa State Univ. Press, Ames, Iowa, USA, 1980.
21. Sooncharernying, S., Edwards, H.M.,Jr.: Aluminum and iron in poultry rations. Georgia Nutrition Conference for the Feed Industry, Atlanta Airport Hilton, Atlanta, Georgia, Nowember 13-15, 1990.

22. Storer, N.L., Nelson, T.S.: The effect of various aluminum compounds on chick performance. Poultry Sci., 1968, 47: 244-247.

23. Hussein, A.S.: The influence of high levels of dietary aluminum on calcium and phosphorus metabolism and performance of chickens and Japanese quail. 1987, Ph.D. Diss., Univ. Ky., Lexington, KY.

24. Berlyne, G.M., Yagil, R., Ben Ari, J., Weinberger, G., Knopf, E., Danovitch, G.M.: Aluminum toxicity in rats. Lancet, 1972, 1: 564-568.

25. Leach, R.M., Burdette, J.H.: Influence of dietary calcium on the pathological lesions associated with endochondral bone formation. Fed. Proc. 46: 887 (Abstr.).

26. Roland, D.A., Sr., Rao, K.S.: New developments concerning phosphorus for commercial Leghorns. Pages 48-67 in: Proceedings Nutrition for the Nineties. Part I. The Science and the Art. Pitman-Moore, Inc., Bloomington, MN, 1990.

27. Cox, G.L., Dodss, M.L., Wigman, H.B., Murphy, F.H.: The effects of high doses of aluminum and iron on phosphorus metabolism. J. Biol. Chem., 1931, 92: 11-12 (Abstr.).

28. Alsmeyer, W.L., Harmon, B.G., Becker, D.E., Jensen, A.H., Norton, H.W.: Effects of dietary Al and Fe on phosphorus utilization. J. Anim. Sci., 1963, 22: 1116 (Abstr.).

29. Peo, E.R., Jr., Lewis, A.J., Grenshaw, J.D., Himmelberg, L.V.: Effect of aluminum on bioavailability of phosphorus from raw rock phosphate for G-F swine. J. Anim. Sci., 1982, 54: 290 (Abstr.).

30. Hussein, A.S., Cantor, A.H., Johnson, T.H., Yokel, R.A.: Effect of dietary aluminum, calcium, phosphorus and sulfate on calcium and phosphorus metabolism and performance of broiler chicks. Poultry Sci., 1987, 66: (Suppl 1): 21 (Abstr.).

31. Szilagyi, M., Bokori, J., Fekete, S., Vetesi, F., Albert, M., Kadar, I.: Effects of long-term aluminum exposure on certain serum constituents in broiler chickens. Eur. J. Clin. Chem. Clin. Biochem. (Germany), 1994, 32, 6: 485-486.