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Effect of Aluminium Toxication on Performance, Egg Quality, Serum Chemistry and Organs of Japanese Quail and Efficacy of Phosphorus Supplementation on Aluminium Induced Alterations

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Abstract: This study was conducted to investigate the toxic effects of various levels of aluminium (Al) on performance, egg quality, blood chemistry as well as organs of laying hens and effects of dietary phosphorus (P) on Al induced alterations in quail. Quail were fed 0, 100, 200, 1000 and 2000 mg kg^{-1} Al in diets or the same levels of Al plus 1.022% P in diets for 6 weeks. None of the Al levels affected live weight, food consumption, feed efficiency and egg weight. At a levels of 100 and 200 mg kg^{-1} Al had no effect on egg production and egg quality. Additional P improved egg specific gravity in 100 and 200 mg kg^{-1} Al groups. Addition of 2000 mg kg^{-1} Al reduced egg production. Additional P increased egg production in 2000 mg kg^{-1} Al group. Egg specific gravity was increased and interior egg quality was decreased in sulphate controls and treatment groups. Additional P had no effect on increased egg specific quality due to high levels of Al. All of Al levels decreased total protein, globulin, total cholesterol levels and ALP activity and increased glucose, Mg, Cu levels and A/G ratio. Serum Pi levels were slightly increased with additional P. Aluminium did not affect kidneys. However, 1000 mg kg^{-1} Al resulted in moderate, 2000 mg kg^{-1} Al resulted in severe fat degeneration in the liver. The effects of additional P on these parameters were variable. In conclusion, especially 2000 mg kg^{-1} Al improved egg shell quality but adversely affected production parameters and caused alterations in the liver and biochemical parameters and additional P had limited effects on these parameters in Japanese quail.

Key words: Aluminium, pathology, performance, phosphorus, quail, serum parameters

INTRODUCTION

Aluminium is normally rarely found in human and animal organisms. But exposure of this element is hardly avoidable because it is an abundant element in the environment (Mahieu *et al*., 2004). Although, nutritionally Al is a nonessential metal (Klein, 1990; Chinoy and Memon, 2001), it is introduced to the body through contaminated food, food supplements or additives as well as water (Nizamlıoğlu *et al*., 1996; Mengi, 1997; Mahieu *et al*., 2004). It is known that Al chemical speciation is a conditioner of its absorption and tissue distribution and accumulation (Mahieu *et al*., 2004). Of particular concern is the effect of Al at the level of the bone, the haematopoietic system and the brain (Cannata-Andia and Martin, 2002; Malluche, 2002; Fyiad, 2007). It has been shown that there is an antagonistic interaction between Al and other minerals, especially P metabolism directly on indirectly thus chronic exposure to Al results in alterations in the bone mineralization (Mahieu *et al*., 2004). In previous studies, the effects of Al on blood chemistry, egg production and egg shell quality has been investigated but conflicting results were reported (Wisser *et al*., 1990; KeshaVarz and Cormick, 1991; Hussein *et al*., 1993; Nizamlıoğlu *et al*., 1996; Kowalczyk *et al*., 2004; Yousef, 2004; Fyiad, 2007). Poultry species are among the most sensitive animals to Al contamination (Nizamlıoğlu *et al*., 1996; Mengi, 1997). Therefore, this study was conducted to investigate the toxic effects of dietary Al on performance, interior and

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exterior egg quality, organs and serum parameters related to carbohydrate, protein, lipid and mineral metabolism. Furthermore to determine the effects of P administrated in combination with Al to minimise the hazardous effects of Al in laying quail.

MATERIALS AND METHODS

Quail, experimental design and diet: Five hundred and twenty eight, 8 weeks old, laying Japanese quail (Coturnix coturnix japonica) were weighed to equal live weights and evenly distributed to 11 groups with 4 replicates consisting of 12 animals each after 10 days of adaptation period. Three control groups were kept. Control I was fed basal diet (diet I) containing 12.21 MJ ME, 18.82% CP and 0.67% P. Quail in treatment groups were fed basal diet (diet I) supplemented with 100, 200, 1000 and 2000 mg kg^{-1} [Al_2 $(SO_4)_3$.18 H_2O] or were fed basal diet (diet II) containing 12.15 MJ ME, 18.78% CP and 1.022% P supplemented with the same levels of Al (Table 1). In the high levels of Al supplemented diets, the sulphate levels were also increased. Therefore, 2 additional control groups were kept and the basal diets (diet I) fed to these groups were supplemented with Na_2SO_4 to provide the same amount of sulphate coming from 1000 and 2000 mg kg^{-1} [Al_2 $(SO_4)_3$.18 H_2O] supplementation. Feed and water were supplied ad libitum. The duration of the study was 6 weeks.

This study was approved by the Ethics Committee of Faculty of Veterinary Medicine, University of Ankara (Approval number: 2001/19).

Table 1: Ingredients and chemical composition of basal diets

	Diets (%)	
	I	II
Ingredients		
Corn	47.34	46.60
Soy bean meal (44% CP)	20.57	20.50
Wheat	10.66	10.15
Full fat soy	6.54	6.47
Meat-bone meal	4.67	5.00
Oil	2.34	2.61
D.C.P.	0.40	2.60
Lime stone	7.03	5.62
Vitamin-mineral mix*	0.20	0.20
Salt	0.25	0.25
Calculated analysis		
Crude protein (%)	18.82	18.78
ME (MJ)	12.21	12.15
Crude cellulose (%)	3.11	3.07
Calcium (%)	3.22	3.24
Phosphorous (%)	0.67	1.08

*2.5 kg of vitamin-mineral mix contains: Vitamin A, 4,000,000 IU; vitamin D3, 480,000 IU; vitamin E, 8,000 mg; vitamin K3, 1 200 mg; vitamin B1, 800 mg; vitamin B2, 2 400 mg; vitamin B6, 1600 mg; vitamin B12, 6 mg, folic acid, 140; cal.d.panth., 2,000 mg; monensin sodium, 40,000 mg; choline chloride, 120,000 mg; nicotin amide, 10,000 smg, Mn, 160,000 mg; Fe, 16,000 mg; Zn, 24,000 mg; Cu, 2,000 mg, I, 160 mg; Co, 40 mg; Se, 60 mg; antioxidant, 4,000 mg

Performance and egg quality measurements: Quails were weighed in the beginning and in the end of the experiments. Egg production was recorded daily and food consumption was recorded at weekly intervals. Feed efficiency was calculated by food consumption (kg)/a dozen of eggs. Twenty eggs from each group were collected to determine of interior and exterior egg quality at weeks 3 and 6. Specific gravity of a whole egg was measured by Archimedes's method at the same day of egg collection. The other egg quality parameters were measured 24 h later. Shell thickness was measured by a micrometer (Mitutoyo, 0.01 mm, Japan). Albumen height, albumen length and width were measured by a compass (Erste, Germany) and then albumen index was calculated. Yolk height and yolk diameter were measured and then yolk index was calculated. Haugh unit was calculated with following formula where the H_A is albumen height and W_E is egg weight (Haugh unit = 100 log H_A + 7.57 - 1.7 $W_E^{0.37}$) (Wells, 1968).

Biochemical measurements: At the end of the experiments, sera were separated by centrifugation at 1300 g after 1 hour incubation at room temperature and stored at -20°C until the analysis. Sera were analysed by a Shimadzu UV Model 1208 using commercial kits for alkaline phosphatase (ALP) (Audit Diagnostica, Ireland), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and glucose, calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg) levels (Biolabo, France), total cholesterol (Biosystem, Spain), total protein and albumin (Chema, Italy). The serum globulin concentrations were calculated by subtracting the albumin values from the total protein values and albumin/globulin ratio was calculated. Serum zinc (Zn) and copper (Cu) levels were determined by Schimadzu flame atomic absorption spectrophotometer.

Histopathological examinations: The pieces of preserved liver and kidney tissues were embedded in paraffin, sectioned 5-6 μm, mounted on glass slides, stained with hematoxylin and eosin (H×E) and examined with a light microscope.

Statistical analysis: Statistical analyses of data were performed by SPSS 9.0 version for Microsoft. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values significant, Duncan's Multiple Range Test was performed. All data were expressed as means±SEMs.

RESULTS

One hundred and 200 mg kg^{-1} Al had no effects on live weight, egg production, food consumption, feed

Table 2: Effects of low levels of aluminium and aluminium+phosphorus on performance of quail

		Al (mg kg^{-1})		Al (mg kg^{-1})+%1.022 P		
Parameters	Control	100 Al	200 Al	100 Al	200 Al	p
Live weight (g)						
Beginning of the experiment	227.06±3.20	225.81±2.62	225.25±2.50	224.50±2.98	227.06±2.53	-
End of the experiment	245.35±3.94	242.06±3.65	240.00±3.22	250.77±3.85	243.03±3.39	-
Food consumption (g)	44.81±1.07	43.77±1.21	45.41±1.48	40.14±1.40	43.97±1.58	-
Egg production (%)	87.00±1.59	86.52±2.46	87.13±2.58	89.06±2.89	81.77±1.39	-
Egg weight (g)	11.76±0.11	12.08±0.14	11.72±0.16	11.99±0.16	11.84±0.19	-
Feed efficiency (kg food/kg egg)	3.81±0.09	3.63±0.12	3.88±0.14	3.35±0.13	3.72±0.13	-

-: not significant

Table 3: Effects of low levels of aluminium and aluminium+phosphorus on egg quality of quail

		Al (mg kg^{-1})		Al (mg kg^{-1})+%1.022 P		
Weeks	Control	100 Al	200 Al	100 Al	200 Al	p
Egg specific gravity (g cm^{-3})						
3	1.0715±0.003	1.0727±0.0009	1.0752±0.0009	1.0738±0.0009	1.0712±0.0010	-
6	1.0709±0.0009^{c}	1.0704±0.0009^{c}	1.0698±0.0011^{c}	1.0745±0.0009^{b}	1.0795±0.0011^{a}	***
Egg shell thickness (mmx10^{-2})						
3	17.85±0.36	17.95±0.41	18.77±0.36	18.11±0.28	18.19±0.35	-
6	18.43±1.38	18.61±0.28	18.50±0.31	18.29±0.37	18.61±0.30	-
Yolk index						
3	50.12±0.74	48.52±0.67	49.67±0.63	49.47±1.06	50.37±0.70	-
6	51.04±0.64	50.24±0.70	50.78±0.54	49.95±1.07	48.93±0.52	-
Albumen index						
3	12.23±0.42	11.41±0.39	11.26±0.25	11.67±0.34	1.12±0.33	-
6	12.12±0.44	11.20±0.44	10.98±0.30	11.37±0.54	10.77±0.35	-
Haugh unit						
3	91.04±0.64	89.01±0.64	89.12±0.53	89.95±0.64	89.34±0.65	-
6	90.43±0.72	88.77±0.83	89.07±0.51	88.36±1.07	87.57±0.77	-

-: Not significant, ***: p<0.001^{a-c}: The mean values within the same row with different superscripts differ significantly

Table 4: Effects of high levels of aluminium and aluminium+phosphorus on performance of quail

				Al (mg kg^{-1})		Al (mg kg^{-1}) + 1.022% P		
Parameters	Control 1	Control 2	Control 3	1000 Al	2000 Al	1000 Al	2000 Al	p
Live weight (g)								
Beginning of the experiment	227.06±3.20	227.09±2.07	226.94±2.20	227.56±2.19	228.31±2.51	228.19±2.40	227.78±2.13	-
End of the experiment	245.35±3.94	240.81±3.69	239.84±3.54	230.00±4.14	235.16±3.95	232.74±4.11	237.19±1.52	-
Food consumption (g)	44.81±1.07	50.08±2.03	50.73±2.61	49.05±2.62	49.52±2.66	50.26±2.93	47.57±2.42	-
Egg production (%)	87.00±1.59^{b}	91.89±0.83^{a}	86.83±0.49^{b}	87.60±1.11^{b}	82.14±0.84^{c}	88.33±1.39^{b}	88.47±0.82^{b}	***
Egg weight (g)	11.76±0.11	11.88±0.07	11.68±0.09	11.66±0.06	11.64±0.14	11.73±0.06	11.42±0.09	-
Feed efficiency (kg food/kg egg)	3.81±0.09	4.21±0.16	4.34±0.20	4.21±0.24	4.25±0.19	4.28±0.24	4.16±0.18	-

-: Not significant, ***: p<0.00; $^{a-c}$: The mean values within the same row with different superscripts differ significantly

efficiency, egg weight and egg quality. Egg specific gravity was increased (p<0.001) in 100 and 200 mg kg^{-1} Al plus P supplemented diet fed groups. And 100 mg kg^{-1} Al plus P supplementation slightly improved feed efficiency (Table 2 and 3). One thousand and 2000 mg kg^{-1} Al had no effects on live weight, food consumption, feed efficiency and egg weight but 2000 mg kg^{-1} Al reduced egg production (p<0.001) and egg production was increased with the P supplementation. Egg specific gravity was increased (p<0.01). Additional P had no effect on increased egg specific gravity due to aluminium. Compared to control I, interior egg quality was decreased (p<0.05, 0.001) by high levels of Al and Al plus P as well as sulphate supplemented diet fed groups (Table 4 and 5).

One hundred and 200 mg kg^{-1} Al decreased serum total protein, globulin (p<0.001), total cholesterol (p<0.05) levels and ALP activity (p<0.001). Both levels of Al had no effect on AST and ALT activities, Ca, Pi and Zn levels but increased albumin (p<0.05), glucose, Cu (p<0.01), Mg, levels and A/G ratio (p<0.001) (Table 6). One thousand and 2000 mg kg^{-1} Al resulted in decreases in total protein, globulin, total cholesterol levels and ALP activity (p<0.001). Glucose, Mg (p<0.01) and Cu (p<0.05) levels as well as A/G ratio (p<0.001) were increased, AST and ALT activities, albumin, Ca, Pi and Zn levels were not influenced by high Al levels (Table 7). The effects of additional dietary phosphorus on these parameters were variable (Table 6 and 7).

In histopathological examinations, high levels of Al had no effect on kidneys. However, in livers remark codons were dissociated and round, sharp edged lipid vacuoles, 1-2 mm and 0.5-1 cm in diameters, were observed

Table 5: Effects of high levels of aluminium and aluminium+phosphorus on egg quality of quail

				Al (mg kg^{-1})		Al (mg kg^{-1}) + 1.022% P		
Weeks	Control 1	Control 2	Control 3	1000 Al	2000 Al	1000 Al	2000 Al	p
Egg specific gravity (g cm^{-3})								
3	1.0715±0.0026^{c}	1.0768±0.0012^{ab}	1.0768±0.0009^{ab}	1.0779±0.0011^{a}	1.0775±0.0011^{a}	1.0729±0.0011^{bc}	1.0752±0.0010^{abc}	**
6	1.0709±0.0009	1.0727±0.0014	1.0715±0.0013	1.0720±1.0011	1.0725±0.0010	1.0722±0.0012	1.0736±0.0011	-
Egg shell thickness (mmx10^{-2})								
3	17.85±0.36	18.80±0.38	18.84±0.27	18.32±0.26	19.31±1.36	18.19±0.32	18.50±0.25	-
6	18.43±1.38	18.79±0.45	18.57±0.28	18.64±0.27	18.77±0.34	18.00±0.32	18.31±0.37	-
Yolk index								
3	50.12±0.74	51.70±0.86	50.66±0.73	51.23±1.03	51.23±1.13	52.15±0.85	51.61±0.89	-
6	51.04±0.64	51.65±1.05	49.26±0.70	50.97±0.55	50.28±0.66	50.12±0.93	48.37±1.16	-
Albumen index								
3	12.23±0.42	11.61±0.59	11.39±0.35	12.00±0.41	11.83±0.39	12.27±0.61	10.52±0.54	-
6	12.12±0.44^{a}	10.25±0.38^{bc}	10.74±0.32^{bc}	11.04±0.42^{ab}	9.63±0.37^{c}	9.62±0.48^{c}	9.99±0.45^{bc}	***
Haugh unit								
3	91.04±0.64^{a}	89.04±1.21^{ab}	88.82±0.69^{ab}	89.12±0.78^{ab}	89.65±0.61^{ab}	90.71±0.96^{a}	87.33±0.67^{b}	*
6	90.43±0.72^{a}	87.39±0.46^{bcd}	87.74±0.64^{bc}	88.20±0.73^{b}	85.43±0.79^{d}	85.88±0.87^{cd}	86.39±0.79^{bcd}	***

-: Not significant; *: p<0.05; **: p<0.01; ***: p<0.001; $^{a-d}$: The mean values within the same row with different superscripts differ significant

Table 6: Effects of low levels of aluminium and aluminium+phosphorus on serum parameters of quail

		Al (mg kg^{-1})		Al (mg kg^{-1}) +1.022% P		
Parameters	Control 1	100 Al	200 Al	100 Al	200 Al	p
Total Protein (g L^{-1})	31.0±1.4^{a}	28.3±1.2^{a}	21.1±1.0^{b}	28.2±0.9^{a}	31.2±1.7^{a}	***
Albumin (g L^{-1})	6.8±0.3^{c}	7.7±0.2^{a}	6.9±0.2^{bc}	7.4±0.2^{ab}	7.5±0.2^{a}	*
Globulin (g L^{-1})	24.3±1.4^{ab}	20.6±1.2^{c}	14.2±0.9^{d}	21.4±0.1^{bc}	24.6±0.8^{a}	***
A/G	0.29±0.07^{c}	0.40±0.03^{b}	0.51±0.03^{a}	0.35±0.01^{bc}	0.31±0.01^{c}	***
Glucose (mmol L^{-1})	14.03±0.53^{c}	14.55±0.48^{c}	15.98±0.22^{ab}	14.92±0.54^{bc}	16.29±0.37^{a}	**
Total Cholesterol (mmol L^{-1})	2.53±0.32^{a}	1.61±0.08^{b}	2.06±0.19^{ab}	2.00±0.14^{ab}	2.43±0.18^{a}	*
ALP (U L^{-1})	914.55±93.43^{a}	199.67±40.97^{c}	140.27±19.96^{c}	170.68±41.46^{c}	389.62±77.31^{b}	***
AST(U L^{-1})	159.39±17.40	147.99±7.54	155.13±13.03	124.99±5.56	119.67±10.67	-
ALT(U L^{-1})	30.20±8.62	30.59±5.73	33.55±6.73	17.13±5.24	15.04±3.87	-
Ca (mmol L^{-1})	5.01±0.20	5.03±0.24	5.07±0.18	5.36±0.19	4.67±0.21	-
Pi (mmol L^{-1})	3.61±0.23	3.83±0.34	3.51±0.15	4.41±0.23	3.95±0.32	-
Mg (mmol L^{-1})	1.14±0.03^{d}	1.50±0.07^{c}	1.99±0.09^{a}	1.39±0.07^{c}	1.75±0.12^{b}	***
Zn (imol L^{-1})	78.03±9.18	64.56±3.98	64.57±6.43	66.10±6.58	62.27±5.05	-
Cu (imol L^{-1})	5.19±0.52^{bc}	6.45±0.52^{a}	5.67±0.30^{ab}	5.82±0.24^{ab}	4.41±0.19^{c}	**

-: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; $^{a-d}$: The mean values within the same row with different superscripts differ significantly

Table 7: Effects of high levels of aluminium and aluminium+phosphorus on serum parameters of quail

		Al (mg/kg)		Al (mg/kg) +% 1.022 P				
Parameters	Control 1	Control 2	Control 3	1000 Al	2000 Al	1000 Al	2000 Al	p
Total Protein (g/L)	31.0±1.4^{c}	34.7±1.7^{ab}	37.6±0.99^{a}	27.0±0.90^{d}	29.7±0.86^{cd}	32.0±0.69^{bc}	28.7±1.0^{cd}	***
Albumin (g/L)	6.8±0.02^{bc}	7.3±0.25^{ab}	7.7±0.37^{ab}	6.6±0.33^{bcd}	6.6±0.20^{bcd}	5.8±0.28^{d}	6.0±0.16^{cd}	***
Globulin (g/L)	24.3±1.40^{cd}	27.4±1.50^{a}	29.8±0.9^{ab}	20.4±0.94^{e}	23.1±0.76^{cde}	26.1±0.60^{bc}	22.7±1.00^{de}	***
A/G	0.29±0.069^{b}	0.28±0.029^{b}	0.26±0.059^{bc}	0.34±0.098^{a}	0.29±0.041^{b}	0.22±0.040^{c}	0.27±0.060^{b}	***
Glucose (mmol/L)	14.03±0.53^{c}	15.87±0.43^{a}	15.58±0.48^{a}	15.23±7.84^{ab}	14.71±0.45^{abc}	15.55±0.41^{a}	13.85±0.31^{c}	**
Total Cholesterol (mmol/L)	2.53±0.32^{a}	2.75±0.23^{a}	3.05±0.24^{a}	2.47±0.10^{ab}	1.83±0.12^{c}	2.07±0.16^{bc}	2.64±0.14^{ab}	***
ALP (U/L)	914.55±93.43^{a}	728.42±110.29^{ab}	942.22±97.85^{a}	515.31±77.07^{bc}	796.87±105.57^{a}	343.99±82.29^{c}	426.89±85.99^{c}	***
AST(U/L)	159.39±17.40	117.11±15.13	142.54±21.05	124.81±27.73	142.46±28.88	114.49±12.18	138.95±17.45	-
ALT(U/L)	30.20±8.62	24.48±6.14	14.83±5.32	10.18±3.02	11.92±3.81	12.48±2.61	25.15±6.88	-
Ca (mmol/L)	5.01±0.20^{bc}	5.23±0.20^{bc}	4.99±0.25^{bc}	4.91±0.28^{c}	4.87±0.15^{c}	5.69±0.19^{ab}	5.94±0.27^{a}	**
Pi (mmol/L)	3.61±0.23	3.96±0.25	3.77±0.23	3.79±0.33	3.28±0.25	4.52±0.37	3.89±0.30	-
Mg (mmol/L)	1.14±0.03^{e}	1.75±0.09^{bc}	1.63±0.09^{cd}	1.43±0.08^{d}	2.08±0.10^{a}	2.03±0.12^{a}	1.99±0.10^{ab}	**
Zn (imol/L)	78.03±9.18	77.88±6.58	98.84±8.72	84.92±6.58	72.52±5.20	75.43±7.65	93.18±9.18	-
Cu (imol/L)	5.19±0.52^{c}	8.18±1.01^{a}	7.87±0.52^{a}	7.71±0.38^{a}	5.98±1.18^{abc}	5.51±0.54^{bc}	7.71±0.74^{ab}	

*-: not significant *: p<0.05 ** : p<0.01 *** : p<0.001; $^{a-e}$: The mean values within the same row with different superscripts differ significantly

in the cytoplasm of hepatocytes in 1000 and 2000 mg kg^{-1} Al groups, respectively (Fig. 1a and 1c). In addition, in 2000 mg kg^{-1} Al group, increases were observed in connective tissue in portal area. Additional dietary P slightly reduced liver fat content in 1000 Al group (Fig. 1b) while had no effect in 2000 Al group (Fig. 1d).

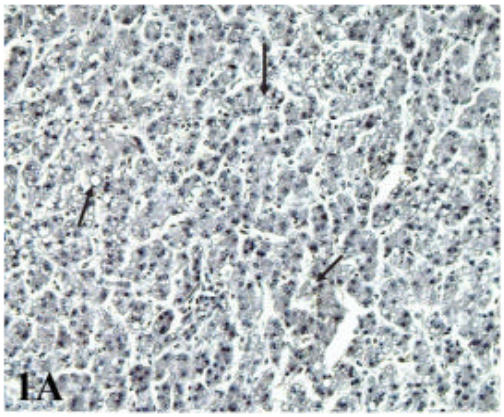

Fig. 1a: Moderate fat degeneration in hepatocytes of liver (1000 Al). H × 200

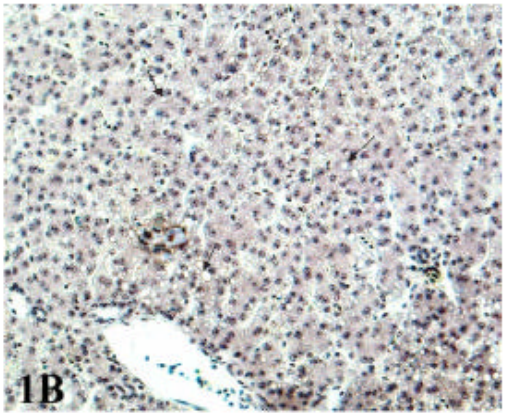

Fig. 1b: Slight fat degeneration in hepatocytes of liver (1000 Al + DCP). H × 200

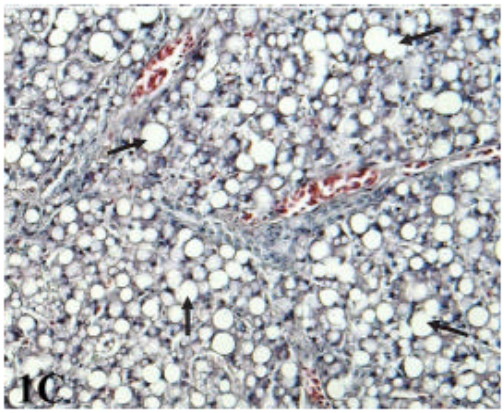

Fig. 1c: Severe fat degeneration in hepatocytes of liver (2000 Al). H × 20

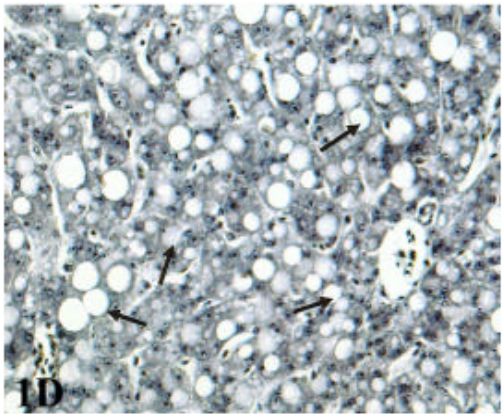

Fig. 1d: Severe fat degeneration in hepatocytes of liver (2000 Al + DCP). H × 200

DISCUSSION

Aluminium a nonessential element is not a nutrient per se. The intake of Al depends on how much Al naturally contaminates the water supply and food, which can be contaminated by Al leached from the soil by acid rain and Al utensils used. Normal free living adults take in an average of 2-5 mg day^{-1} with food. The quantity of Al received by human may range from at least 10-6300 μg L^{-1} by various contaminations (Klein, 1990). Although, the amount of Al used in this study may not be taken by animals through natural contaminations, the high levels were applied to induce a severe toxication in animals for determination of the efficacy of phosphorus supplementation to alleviate the hazardous effects of aluminium.

Previous studies have reported that the adverse effects of Al on performance are variable depending on the levels of Al contamination (Hussein *et al*., 1993; Firat and Tekeli, 1997; Capdevielle *et al*., 1998; Kowalczyk *et al*., 2004). In the present study, 100 and 200 mg kg^{-1} Al had no effects on live weight, food consumption, feed efficiency, egg production as well as egg weight and interior and exterior egg qualities. It was reported that Al adversely affected live weight, production, food consumption and feed efficiency (Fethiere *et al*., 1990; Roland *et al*., 1990; Wisser *et al*., 1990; Kowalczyk *et al*., 2004). However, food consumption was slightly increased and feed efficiency was also slightly affected in 1000 and 2000 mg kg^{-1} levels of Al as well as in the control groups that fed diets supplemented with the same amount of sulphate as in the study of Zohouri *et al*. (1998). Thus, increased food consumption and worsening feed efficiency may result from not only Al per se but also from high sulphate content of the diets.

A significant decrease in egg production in 2000 mg kg^{-1} Al supplemented diet fed group confirmed the results of the previous studies conducted with similar or higher Al levels (Hussein *et al*., 1989; Fethiere *et al*., 1990; Nizamlıoğlu *et al*., 1998). The decreased egg production was reached to the egg production levels of the control group with the increasing P levels of the diet. Thus, it can be speculated that the P levels of the diet may play a role in prevention of the adverse effects of Al on egg production.

Interior egg quality was adversely affected in both 1000 and 2000 mg kg^{-1} Al and sulphate supplemented diets fed groups. Alterations in interior egg quality may results from either Al itself or sulphate content of $AlSO_4$ or both. High levels of Al significantly increased egg specific gravity and slightly increased egg shell thickness at week 3. The improvement in egg shell quality by high levels of Al was consistent with the results of previous studies (Roland, 1988).

It was reported that Al, in the organism, may be diverted into soluble forms such as citrate, sulphate and nitrate salts but mostly are diverted into insoluble aluminium phosphate forms which prevent the absorption of phosphate thus decreases serum Pi levels (Hussein *et al*., 1989; Rossi *et al*., 1990; Hussein *et al*., 1993). On the other hand, no effects of Al on serum Pi levels were also reported (Wisser *et al*., 1990; Keshavarz and Cormick, 1991) consistent with the results of this study. Inconsistent results were reported concerning the effects of Al on serum Ca levels. Some researchers found decreases (Nybo, 1996; Capdevielle *et al*., 1998), others found increases in serum Ca (Nizamlıoğlu *et al*., 1996) while some others found no effects of Al on serum Ca levels (Hussein *et al*., 1989; Keshavarz and Cormick, 1991; Hussein *et al*., 1993). It was reported that high levels of Al adversely affected skeletal development in layers (Capdevielle *et al*., 1998). Aluminium reduced serum and bone Zn and Mg levels (Johnson *et al*., 1992; Nizamlıoğlu *et al*., 1996). About 94% of the egg shell is calcium carbonate, 3% of the egg shell is organic matters and the remaining of it consist of MgO, PO_5, water and some other minerals. In the formation of egg shell, Mg and Zn are also important as well as Ca and Pi (Özpınar, 1986). Some studies reported that Al reduced serum and bone Zn and Mg levels. However, in the present study, serum Ca, Zn and Pi levels were not influenced by any levels of Al. But serum Mg and Cu levels were increased in both Al and sulphate supplemented diet fed groups. Thus these increases may results from sulphate rather than Al. Increased dietary P levels tended to return serum Cu levels similar to control levels in the group fed 1000 mg kg^{-1} Al.

Aluminium can severely affect the energy output and thus can lead to a variety of cellular abnormalities (Mailloux and Appanna, 2007). In all Al supplemented groups, decreases in total protein and globulin levels and increases in A/G ratio may suggest the altered protein metabolisms as indicated previously (Chinoy and Memon, 2001; Fyiad, 2007; Mailloux and Appanna, 2007) and except in 2000 mg kg^{-1} Al group, increased dietary P may slightly prevent adverse effects of Al. Aluminium decreased ALP activity in all groups; the other serum enzymes were not influenced. Kowalczyk *et al*. (2004) also showed that serum AST, ALT and glucose levels were not affected by $AlCl_3.6H_2O$ in experimental animals. Furthermore, Szilagyi *et al*. (1994) reported no effects of Al on glucose in broilers. In contrast, in the present study the increased serum glucose levels in all Al supplemented groups may reflect the impairment in carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by an increase in adrenocorticotrophic and glucagon hormones and /or reduced insulin activity (Yousef, 2004; Fyiad, 2007). The increases in glucose levels may result from the depression of glycolysis due to inhibition of hexokinase as well as formation and accumulation of enzymatic decarboxylation of pyruvic acid and inhibition of the conversion of isocitric acid to á-ketoglutaric acid (Mengi, 1997). The serum total cholesterol levels were decreased by all Al levels in the present study as indicated by Fœrat and Tekeli (1997). In the present study, dose dependent increases in size of fat droplets in the cytoplasm of hepatocytes were observed by histopathological examinations. Findings of histopathological examination showed that 1000 mg kg^{-1} Al caused moderate, 2000 mg kg^{-1} Al severed fatty liver. The increased dietary P level decreased the severity of the fatty liver in 1000 mg kg^{-1} Al supplemented group but did not affect in 2000 mg kg^{-1} Al supplemented group. The fat degeneration in the liver confirms the alterations in serum parameters (Başoğlu and Sevinç, 2004).

CONCLUSION

The results of this study have shown that the adverse effects of aluminium provided from $AlSO_4$ may result from Al per se as well as high sulphate and phosphorus level supplemented in this study could partly prevent the adverse effects of aluminium in Japanese quail.

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