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# PHYTASE REDUCES CADMIUM INTOXICATION IN JAPANESE QUAILS

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## ABSTRACT

One hundred eighty 14-d old Japanese quail chicks were individually weighed and randomly distributed into six treatments with three replicates, 10 chicks each. The chicks were reared up to 42 days of age. Cadmium (Cd) in the form of cadmium chloride ($CdCl_2.2½\ H_2O$) was added to the basal diet at 0, 5 and 50 mg/Kg diet with or without 1000 units of phytase supplementation. The results indicated that there was marked reduction in body weight and feed consumption and worse feed conversion ratio for birds fed 50 ppm Cd while slightly reduction was noticed for those received 5ppm compared with the control group. Addition of phytase alleviate completely and partially the adverse effect of 5 and 50 ppm Cd on growth performance, respectively. Linear significant reduction was observed for hematocrit, hemoglobin, plasma concentration of total protein and its fractions, Ca, and P in quail chicks fed Cd contaminated diets comparable to those of control and phytase groups. Conversely, A/G ratio, uric acid, creatinine, AST and ALT were increased, indicating hepatic and renal dysfunction. Supplemental phytase in combination with Cd could eliminate the adverse effect of Cd, depending upon the dose inoculated. Chicks whose diets polluted with either 5 or 50 ppm Cd showed lower humoral immune response at primary and secondary antibody titers than those of control and phytase groups. Therefore, chicks of 5 ppm Cd in blend with phytase was insignificantly different from those of Cd free diets. Significant lower dressing and relative tibia weight was noted for birds exposed to Cd either at low or high level as compared with those of control, phytase and 5 ppm Cd in combination with phytase. Opposite trend was noted for the relative weights of liver, kidney, heart, gizzard and brain. A wide range of histological degenerations and damages were observed in the hepatic and renal tissues of Cd exposed chicks. The magnitude of these lesions was dose dependent. However, normal histological appearance was almost noted as phytase combined with 5 ppm Cd.

It could be concluded that phytase supplementation reduce the adverse effect of cadmium on growth performance, hematological and physiological aspects and immune response. However, more researches are needed to determine the optimal level of phytase under different levels of cadmium contamination to achieve normal productive and physiological status of Japanese quails.

**Keywords:** quail, cadmium, phytase, growth performance, blood constituents, immune, histology.

## INTRODUCTION

Cadmium is a nonessential and strongly toxic element. If absorbed into the organism through the digestive and pulmonary systems, cadmium forms complexes with proteins, in which it is easily transported and stored, mainly in the liver and kidneys and, in smaller quantities, in the pancreas, intestines and bones. Cadmium is also responsible for upsetting the activity of a number of cellular enzymes (Króliczewska and Dach, 1997 and Alloway and Ayres, 1999)

It is present as a contaminant in food (leafy vegetables, grains, and cereals), water, and tobacco leaves, as well as being a by-product of zinc and

lead mining and smelting. Approximately 20 to 50 million metric tons of electronic wastes which includes cadmium are generated worldwide every year as a result of the growing demand for computers, mobile phones, TVs, radios and other consumer electronics. Because of its widespread nature, cadmium can either be ingested via contaminated foods or inhaled. Even in small amounts (~200 ppm) significant damage occurs to the kidneys and to the gastrointestinal tract (Bremner, 1978). Other manifestations of cadmium toxicity include mild anemia and osteoporosis. The most pronounced effects occur in the kidney. Only when significant cadmium damage such as proteinuria and decreased renal function has occurred does significant cadmium excretion occur. Also, cadmium is able to alter the immune function (Sant'Ana *et al*., 2005) where it could suppress humoral and cell mediated immunity of broiler chickens (Vodela *et al*., 1997). Unfortunately, there are few if any symptoms of chronic exposure to small amounts of cadmium. The most dangerous characteristic of cadmium is that it accumulates throughout a lifetime.

Furthermore, cadmium interacts with the metabolism of four metals essential to nutrition: zinc (Zn), iron (Fe), calcium (Ca) and copper (Cu) (Goyer, 1995 and Berzina *et al*., 2007). Another important nutritional aspect of cadmium is its interaction with the metallothionein (MT) which is a cysteine-rich protein present in many living organisms and has a potential role into detoxification of nonessential heavy metals i.e., cadmium and mercury (Martínez *et al*., 2004). These and other factors are important in determining the susceptibility of an animal to cadmium toxicity.

Several previous researches have been conducted to eliminate the harmful effect of cadmium through single or combination of different elements. Vodela *et al*. (1997), Martínez *et al*. (2004) and Jacquillet *et al*. (2006) concluded that the single addition of certain element i.e., Zn, Fe or Ca, could partially overcome the unfavorable cadmium toxicity effects. However, using a mixture of different elements or a product that has the potentiality to provide adequate amount of these minerals may be more efficient in evoking the tolerance to cadmium intoxication.

Thus, supplemental microbial enzyme phytase can improve the apparent absorption of metals essential to nutrition such as calcium, zinc, copper and iron while lowering liver and kidney cadmium accumulation (Rimbach *et al*., 1995). The addition of microbial phytase counteracted the decrease in growth and zinc status brought on by cadmium.

The objective of the present study was to investigate the efficiency of phytase enzyme to minimize the cadmium toxicity effects on growth performance, physiological and histological aspects, and immune response of growing Japanese quails.

## MATERIALS AND METHODS

The present study was carried out at Quail Production Unit, Agricultural Experiment and Researches Unit, Faculty of Agriculture, Ain Shams University.

One hundred eighty 14-d old Japanese quail chicks were individually weighed and randomly distributed into six treatments with three replicates, 10

chicks each. The chicks were reared up to 42 days of age in electrically heated, batteries and maintained on a 24-h constant light schedule. Feed and water were provided *ad-libitum*.

The basal corn-soybean meal diet (Table 1) was formulated to meet the requirements of starting and growing chicks through the experimental period (NRC, 1994). Cadmium (Cd) in the form of cadmium chloride ($CdCl_2.2^1/_2\ H_2O$) was added to the basal diet at 0, 5 and 50 mg/Kg diet with or without 1000 units (U) of phytase supplementation (Natophous). Individual body weight (BW), body weight gain (WG), feed consumption (FC) and feed conversion ratio (FCR) were recorded biweekly intervals. Mortality maintained weekly.

**Table 1. Composition and calculated analysis of the experimental diet.**

| **Ingredient** | **%** |
|---|---|
| Yellow corn | 55.20 |
| Soybean meal (44%) | 36.00 |
| Corn gluten (60%) | 5.80 |
| Bone meal | 1.22 |
| Plant oil | 0.15 |
| Limestone | 0.86 |
| Methionine | 0.104 |
| Lysine | 0.125 |
| Premix* | 0.30 |
| Salt | 0.25 |
| Total | 100 |
| **Calculated analysis** | |
| Crude protein % | 24.012 |
| Metabolizable Energy (kcal/kg) | 2906.16 |
| Crude fiber % | 3.86 |
| Crude fat % | 2.44 |
| Calcium (%) | 0.8 |
| Available phosphorus (%) | 0.3 |

***Each kilogram of diet contains = A, 12000 I.U., D3, 2500 I.U., E, 10mg., B1, 2mg., B2, 5mg., B6, 4mg., B12, 10µg., Niacin, 25mg., Pantothenic acid, 10mg., Biotin, 50µg., Folic acid, 1000µg., and Choline chloride, 255mg. Selenium, 300µg., Copper, 10mg., Iodine, 1.0mg., K, 2.0mg., Iron, 33mg., Manganese, 60mg.and , 60mg. Zinc.**

To determine the primary and secondary humoral immune response, chicks in one replicate for each treatment, at 4 and 5 wk of age were injected intramuscularly with 0.5 ml of 40% saline suspension of SRBC's for determining the antibody responses. Blood samples were collected from each bird seven d after SRBC's challenge. The total, mercaptoethanol-sensitive (MES, presumably Ig M) and mercaptoethanol-resistant (MER, presumably Ig G) anti-SRBC antibody titers were determined using a micro-heamagglutination technique as described by Yamamoto and Glick (1982) and Dix and Taylor (1996). The antibody data were expressed as the log2 of the reciprocal of the highest dilution giving visible agglutination.

At 6 wk of age, randomly 15 birds from each treatment were individually weighed then slaughtered by severing the carotid artery and jugular veins and autopsied. Dressing, heart, liver, kidney, gizzard, tibia, spleen and bursa were measured as a percentage of live body weight. Blood samples were collected at slaughtering in heparinized tubes. Sample of

collected blood was used for hematological parameters determination. Plasma was separated by centrifugation of the rest sample at 4000 rpm for 15 min and stored at -20°C until assay. The following biochemical analyses were conducted using commercial kits according to the procedure outlined by manufacturer: plasma total protein, albumin, uric acid, creatinine, aspartate aminotransferase (AST), alanine amino transferase (ALT)and glucose (Diamond Diagnostic, Cairo Egypt.), calcium and phosphorus (Spinreact, S.A. Spain). Globulin was calculated by subtraction of plasma albumin from total protein. Samples of kidney and liver were taken, fixed in 10% formalin saline, dehydrated, paraffin embedded, sectioned and stained with hematoxilin and eosin. Then sections were examined microscopically.

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 1994). Differences among means were tested using Duncan's multiple range test (Duncan, 1955). Percentages of slaughter traits were divided by 100 and subjected to arc sin transformation of the square root before analysis; however actual percentage means are presented.

## RESULTS AND DISCUSSION

### Growth Performance

The effect of cadmium (Cd) and phytase supplementation on growth performance parameters is shown in Table 2. The overall results indicated that, there was slightly decrease (6.4%) in BW and WG in birds exposed to 5 ppm Cd while the reduction was more pronounced (21%) with those fed 50 ppm compared with the control group. Similar results were obtained by Berzina *et al*. (2007) who reported that growth retardation (by 27%) was occurred in chicken fed the cadmium-enriched diet. Addition of phytase improved BW and WG by about 6.6, 4.2 and 16.4% for those received 0, 5 and 50 ppm Cd, respectively, compared with unsupplemented ones. Therefore, the results of BW and WG were approximately similar either between the group fed 5 ppm Cd plus phytase and control group or those administrated 50 ppm Cd plus phytase and 5 ppm Cd. The results of feed consumption followed the same trend of BW where the heavier groups consumed more food compared with the lighter ones. In this respect, birds received high level of Cd (50ppm) recorded the lowest value of FC while, those fed control diet plus phytase gave the highest value. The results of FCR indicated that, the group administrated 50 ppm Cd gave markedly the worst value while group fed control diet gave the best one. Also, there were no significant differences in FCR between the groups received 5 ppm Cd with or without phytase supplementation and those fed control or 50 ppm Cd plus phytase diets. The results agree with the reports mentioned that Cd decreased feed consumption (Lisunova *et al*., 2006) and lowered feed efficiency (Erdogan *et al*., 2005). Mortality rate was in the normal values and there were no differences between the different groups.

From the results obtained it would be noticed that, under our experimental conditions, Japanese quail chicks exhibited tolerance to Cd at 5 ppm with slightly effects on growth performance parameters while 50 ppm Cd appeared to be a toxic level. The adverse effect of Cd on growth performance

may be due to the antagonistic relationships between this metal and some micronutrients specially zinc and iron. Cadmium has an inhibitory effect on the activity of zinc-containing enzymes such as carboxypeptidase (Coleman and Vallee, 1961) and -mannosidase (Snaith and Levvy, 1969).

**Table 2: Effect of phytase (Ph) supplementation on growth performance of quail chicks during cadmium (Cd) intoxication.**

| Age (wk) | Treatments | | | | | | SEM | Sig. |
|---|---|---|---|---|---|---|---|---|
| | Control | Control + Ph | Cd 5 ppm | Cd 50 ppm | Cd 5 ppm + Ph | Cd 50 ppm +Ph | | |
| | **Body weight, gm** | | | | | | | |
| **2** | 54.86 | 54.93 | 55.68 | 56.19 | 55.67 | 55.10 | 2.51 | NS |
| **4** | 128.52$^{b}$ | 136.85$^{a}$ | 119.48$^{c}$ | 99.84$^{d}$ | 124.29$^{b}$ | 116.57$^{c}$ | 3.46 | * |
| **6** | 186.75$^{b}$ | 199.10$^{a}$ | 174.82$^{c}$ | 147.40$^{d}$ | 182.11$^{b}$ | 171.50$^{c}$ | 3.84 | * |
| | **Weight gain, gm** | | | | | | | |
| **2-4** | 73.66$^{b}$ | 81.92$^{a}$ | 63.8$^{c}$ | 43.65$^{d}$ | 68.62$^{c}$ | 61.47$^{c}$ | 3.12 | ** |
| **4-6** | 58.23$^{b}$ | 62.25$^{a}$ | 55.34$^{b}$ | 47.56$^{c}$ | 57.82$^{b}$ | 54.93$^{b}$ | 3.79 | * |
| **0-6** | 131.89$^{b}$ | 144.17$^{a}$ | 120.14$^{c}$ | 91.21$^{d}$ | 126.44$^{bc}$ | 116.40$^{c}$ | 4.03 | ** |
| | **Feed consumption, gm** | | | | | | | |
| **2-4** | 235.71$^{b}$ | 257.22$^{a}$ | 211.82$^{c}$ | 171.47$^{d}$ | 220.27$^{c}$ | 205.92$^{c}$ | 13.62 | ** |
| **4-6** | 238.74$^{a}$ | 238.42$^{a}$ | 231.87$^{a}$ | 210.69$^{b}$ | 238.22$^{a}$ | 233.40$^{a}$ | 19.01 | * |
| **2-6** | 474.45$^{ab}$ | 495.64$^{a}$ | 443.69$^{c}$ | 382.16$^{d}$ | 458.49$^{bc}$ | 439.32$^{c}$ | 23.37 | * |
| | **Feed conversion ratio** | | | | | | | |
| **2-4** | 3.20$^{a}$ | 3.14$^{a}$ | 3.32$^{b}$ | 3.93$^{c}$ | 3.21$^{a}$ | 3.35$^{b}$ | 0.09 | * |
| **4-6** | 4.10$^{b}$ | 3.83$^{a}$ | 4.19$^{bc}$ | 4.43$^{d}$ | 4.12$^{b}$ | 4.25$^{c}$ | 0.12 | ** |
| **2-6** | 3.59$^{ab}$ | 3.43$^{a}$ | 3.69$^{bc}$ | 4.19$^{d}$ | 3.62$^{bc}$ | 3.77$^{c}$ | 0.15 | * |

**$^{a-d}$ Means within the same row with different letters are significantly differed. NS=Non Significant * P≤0.05 **P≤0.01**

Indeed, cadmium replaces zinc in MT (Bremner, 1978). There are claims of activity reductions of other zinc-containing enzymes that could be pertinent to the toxicity associated with cadmium. The toxicity of cadmium may result from disturbances in zinc metabolism, leading to the description of cadmium as an antimetabolite of zinc (Cotzias and Papavasiliou, 1964). Furthermore, some of the symptoms of chronic cadmium toxicity are similar to those of zinc deficiency (Underwood, 1977) as growth failure (Bunn and Matrone, 1966) for example. The poor growth performance associated with high level of cadmium could be also attributable to the interference of cadmium with iron absorption at the intestinal level. Cadmium binds to liver ferritin, which is also present in the intestinal mucosa and involved in the mucosal uptake and transfer of iron. It has been suggested that higher gastrointestinal absorption of cadmium is due to lower body iron stores as measured by the concentrations of serum ferritin (Peraza *et al*., 1998).

The role of phytase to elevate the adverse effect Cd could be explained by its indirect effect on releasing many of micronutrients from phytic acid which counteract with the absorption Cd such as zinc and iron. Many researches support this hypothesis (Ao *et al*., 2007; Jondreville *et al*., 2007)

**Hematological Parameters**

The effect of Cd, phytase and their combination on hematological and blood biochemical parameters is listed in Table (3). It is clearly observed that hematocrit (Ht) and hemoglobin (Hb) values followed similar trend as

they were greatly (p<0.001) reduced in quail chicks exposed to Cd contaminated diets. These results confirmed those reported in broiler by El-Sebai *et al*. (1994) and Abaza (1996) and Zein El-Dein *et al*., (2000). The harmful effect of Cd induced anemia could be due to the interference of cadmium with iron metabolism and absorption at the intestinal level. Thus, Cd binds to intestinal mucosa of both ferritin, which involved in the uptake and transfer of iron, and transferrin, which donates iron to the heme during hemoglobin synthesis pathway (Peraza *et al*., 1998).

**Table 3: Effect of phytase (Ph) supplementation on some blood constituents in quail chicks during cadmium (Cd) intoxication.**

| Variable | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Control + Ph | 5ppm Cd | 50ppm Cd | 5ppm Cd+ Ph | 50ppm Cd+ Ph | SEM | Sig. |
| Hematocrit (Ht) | 40.60$^{a}$ | 41.40$^{a}$ | 36.40$^{c}$ | 27.80$^{e}$ | 38.80$^{b}$ | 32.60$^{d}$ | 1.14 | *** |
| Hemoglobin, g/dl | 11.44$^{a}$ | 11.72$^{a}$ | 9.00$^{c}$ | 5.00$^{e}$ | 10.12$^{b}$ | 7.16$^{d}$ | 0.49 | *** |
| Total protein, g/dl | 3.95$^{a}$ | 4.04$^{a}$ | 3.43$^{b}$ | 3.06$^{c}$ | 3.82$^{a}$ | 3.12$^{c}$ | 0.04 | *** |
| Albumin, g/dl | 1.68$^{abc}$ | 1.78$^{a}$ | 1.70$^{ab}$ | 1.59$^{c}$ | 1.73$^{a}$ | 1.61$^{bc}$ | 0.01 | ** |
| Globulin, g/dl | 2.27$^{a}$ | 2.26$^{a}$ | 1.73$^{b}$ | 1.47$^{c}$ | 2.10$^{a}$ | 1.51$^{bc}$ | 0.03 | *** |
| A/G ratio | 0.75$^{b}$ | 0.79$^{b}$ | 0.98$^{a}$ | 1.08$^{a}$ | 0.83$^{b}$ | 1.07$^{a}$ | 0.01 | ** |
| Glucose, mg/dl | 221$^{a}$ | 199$^{ab}$ | 171$^{bc}$ | 155$^{c}$ | 195$^{ab}$ | 167$^{bc}$ | 11.3 | ** |
| Ca, mg/dl | 9.59$^{a}$ | 9.99$^{a}$ | 9.05$^{a}$ | 6.33$^{c}$ | 9.34$^{a}$ | 7.39$^{b}$ | 0.36 | *** |
| P, mg/dl | 6.75$^{ab}$ | 7.52$^{a}$ | 6.34$^{b}$ | 3.98$^{d}$ | 6.87$^{ab}$ | 5.22$^{c}$ | 0.03 | *** |
| Uric acid, mg/dl | 4.14$^{cd}$ | 3.91$^{d}$ | 4.45$^{bc}$ | 5.34$^{a}$ | 4.25$^{cd}$ | 4.78$^{b}$ | 0.14 | ** |
| Creatinine, mg/dl | 0.71$^{d}$ | 0.63$^{d}$ | 0.89$^{c}$ | 1.91$^{a}$ | 0.70$^{d}$ | 1.49$^{b}$ | 0.00 | *** |
| AST, U/ l | 20.70$^{c}$ | 20.00$^{c}$ | 21.45$^{c}$ | 26.55$^{a}$ | 21.37$^{c}$ | 24.01$^{b}$ | 1.26 | *** |
| ALT, U/ l | 100$^{d}$ | 94$^{d}$ | 107$^{c}$ | 125$^{a}$ | 101$^{cd}$ | 118$^{b}$ | 5.43 | *** |

**$^{a-d}$ Means within the same row with different letters are significantly differed. NS =Non Significant ** P≤0.01 *** P≤0.001**

The current results showed that supplemental phytase in combination with Cd could markedly correct the anemia caused by cadmium exposure. These imply that phytase may improve iron utilization and absorption in the gastrointestinal tract. Pintar *et al*. (2005) found that tibia iron content was significantly increased when broilers fed diets containing 1000 PU/kg. They ascribed this result to the high phytase activity.

**Blood Components**

Compared with both control and phytase chicks, plasma concentration of total protein and its fractions were significantly decreased but A/G ratio increased, in quail chicks fed diets contaminated with either 5 or 50ppm Cd. This finding coincided with those of Fathi *et al*. (1999), Zein El-Dein *et al*. (2000) and Herzig *et al*. (2007). The decrease in blood total protein is an indicator of impaired protein synthesis and has been observed in chicks suffering from heavy metals (Khan *et al*., 1993). Similar trend was noted for plasma glucose level, moreover the effect was more pronounced with the higher dose of Cd (50ppm). These results could be account for the occurrence of sever nephropathy due to Cd contamination (Aughey *et al*., 1984 and Brzoska *et al*., 2003). This nephrotoxicity cause reabsorptive and secretory dysfunction of the renal tubule through generating free radicals and

by inducing necrosis and apoptosis. The main signs include proteinuria, ion losses, glucosuria, aminoaciduria and polyuria (Jacquillet *et al*., 2006).

The most noteworthy findings of those results are that the effect of co-treatment with 1000 U/kg phytase, during 5 ppm of Cd administration, completely prevented the unfavorable changes in the aforementioned blood components. However, this beneficial effect of phytase was slightly established with the higher level of Cd (50ppm). Rambeck and Walther (1993) reported that dietary supplementation of microbial phytase has been shown to reduce Cd accumulation in Japanese quail.

As it was expected, severe and linear reductions were detected in plasma concentration of both calcium and phosphorus as a result of Cd intoxication. This could be due to cadmium has an inhibitory effect on intestinal calcium transport that is stimulated by vitamin D (Ando *et al*., 1981). Also, Cd induces hypercalciuria and hyperphosphouria through either its interaction with the calcium and phosphorus reabsorption process or its toxic actions against the renal tubules in general (Peraza *et al*., 1998). In agreement with the present results, Zein El-Dein *et al*. (2000) reported significant decreases in plasma calcium and phosphorus concentrations of broiler chicks fed on diets contaminated with 40, 80 or 120ppm Cd.

Due to the action of phytase in the availability of minerals and trace elements like calcium, zinc, phosphorus and iron, which are known to reduce the bioavailability of cadmium in different animal species (Guillot and Rambeck, 1995; Ghasemi *et al*., 2006 and Jondreville *et al*., 2007). Supplemental 1000 U/kg phytase could fully and moderately eliminated the destructive effect of Cd inclusion at 5 ppm and 50 ppm, respectively on plasma level of both Ca and P.

Japanese quail chicks exposed to Cd at either 5 or 50 ppm showed renal dysfunction, in which they had higher plasma level of uric acid and creatinine than those of control with or without phytase and 5 ppm Cd plus phytase. Nomiyama and Nomiyama (1984) reported in rabbit that Cd caused renal dysfunction, as it increased excretion of protein (proteinuria), aminoaciduria and decreased creatinine clearance. On the other hand, Erdogan *et al*. (2005) reported that Cadmium ingestion did not alter serum creatinine levels in broilers. Regarding liver functions, it could be noticed a significantly higher level of AST and ALT in Cd treated groups compared to control and phytase groups. That indicated the Cd contamination caused liver dysfunction. These results are in agreement with those of Fathi *et al* (1999) and Zein El-Dein *et al*. (2000). However, Erdogan *et al*. (2005) who found that Liver function enzymes, AST and ALT activities were not changed by cadmium. Provision of phytase to Cd polluted diets tended to adjust the liver dysfunction; however the supplemental dose of phytase may be not sufficient to completely remove the deleterious effects of Cd on liver function.

**Immune Response**

Sheep red blood cells (SRBC's) are thymic-dependent antigens that require T cells, B cells, and cytokines for antibody synthesis. From table (4) it clearly observable that, total antibodies production and IgM (MES) estimated seven d post primary challenge with SRBC's were significantly decreased in

quail chicks exposed to 50 ppm Cd. Additionally, supplemental 1000 U/kg phytase in combination with 50 ppm failed to promote the primary immune response. On the other hand, an enhancement was achieved when chicks received 5 ppm Cd in combination with phytase, as they did not significantly differ from those of control and phytase groups.

Chicks whose diets was contaminated with either 5 or 50 ppm Cd, whether singly or in blend with phytase recorded significantly lower total antibodies titer, determined seven d post secondary immunization with SRBC's, comparable to those of control and phytase treatments. Moreover, the reduction was Cd dose dependent. Similar trend was found to the IgG (MER) antibodies, except that the chicks of 5 ppm Cd in combination with phytase was insignificantly different from those of Cd free diets.

**Table 4: Effect of phytase (Ph) supplementation on immune responses and relative weights of bursa and spleen in quail chicks during cadmium (Cd) intoxication.**

| Treatment | Primary Antibody Titers | | | Secondary Antibody Titers | | | Lymphoid Organs | |
|---|---|---|---|---|---|---|---|---|
| | Total | IgM | IgG | Total | IgM | IgG | Bursa | Spleen |
| Control | 3.73$^{a}$ | 2.91$^{a}$ | 0.82 | 5.45$^{ab}$ | 1.27 | 4.18$^{a}$ | 0.09$^{d}$ | 0.051 |
| Control+Ph | 3.64$^{a}$ | 2.73$^{ab}$ | 0.91 | 5.63$^{a}$ | 1.09 | 4.54$^{a}$ | 0.10$^{cd}$ | 0.052 |
| 5ppm Cd | 3.09$^{bc}$ | 2.00$^{c}$ | 1.09 | 4.45$^{c}$ | 1.36 | 3.09$^{b}$ | 0.11$^{bc}$ | 0.061 |
| 50ppm Cd | 2.54$^{d}$ | 1.91$^{c}$ | 0.64 | 3.54$^{d}$ | 1.18 | 2.36$^{c}$ | 0.14$^{a}$ | 0.071 |
| 5ppm Cd+ Ph | 3.50$^{ab}$ | 2.70$^{ab}$ | 0.80 | 5.10$^{b}$ | 1.10 | 4.00$^{a}$ | 0.10$^{cd}$ | 0.058 |
| 50ppm Cd+ Ph | 2.90$^{cd}$ | 2.20$^{bc}$ | 0.70 | 4.30$^{c}$ | 1.10 | 3.20$^{b}$ | 0.12$^{ab}$ | 0.067 |
| SEM | 0.071 | 0.041 | 0.003 | 0.035 | 0.004 | 0.007 | 0.002 | 0.002 |
| Sig. | *** | ** | NS | *** | NS | *** | *** | NS |

**$^{a-d}$ Means within the same column with different letters are significantly differed. NS=Non Significant ** P≤0.01*** P≤0.001**

The current results are in accordance with those of Vodela *et al*. (1997) who postulated that exposure of broiler chickens to chemical contaminates including Cd in drinking water resulted in significant suppression of humoral and cell mediated immunity at 21 and 49 d of age. They attributed the suppression of antibody formation during heavy metals contamination to the impairing T helper lymphocyte functions as well as the greater reduction of vitamins and minerals which are responsible for better immune response. On the other hand, Sant'Ana *et al*. (2005) claimed that kidney function and cellular immune response were not affected in Japanese quail exposed to 100 ppm Cd form 1-28 d old.

The improved humoral immune response against Cd contamination attained by supplemental phytase may be ascribed for the higher bioavailability of minerals and trace elements like zinc, iron and selenium, in addition to increasing induction of which are known to boost the birds' immunocompetence (Guillot and Rambeck, 1995). In addition, increasing the induction of Metallothionein (MT) is a low-molecular-weight (7 kDa) cysteine-rich protein present in many living organisms This protein has been suggested to several functions include detoxification of nonessential heavy metals (cadmium and mercury), metal transfer, free-radical scavenger, and metal storage (Martínez *et al*., 2004).

With respect to the lymphoid organ relative weights (bursa and spleen) the present results illustrated highly significant increase in the relative weights of bursa in Cd treated groups either alone or in blend with phytase. Similar results was observed for the spleen relative weights, however the differences among groups were not statistically significant.

These results are in full agreement with those of Vodela *et al*. (1997) who found that, increasing concentrations of contaminants, including Cd, in drinking water resulted in linearly ($P$<0.01) increasing relative bursa weights, but spleen did not alter in broiler chickens. They added that the observed increase in bursa relative weight was microscopically associated with lymphoid depletions and thinning of follicular cortices or depletion of lymphocytes in the medulla. Also, Zein El-Dein *et al*. (2000) reported similar aforementioned results for broiler chicks fed diets contaminated with 40, 80 or 120 ppm Cd. The hypertrophy of bursa could be attributed to the toxic effects of Cd; consequently the dietary Cd may lead to depression of immune response of quail chicks as previously reported herein for the hemagglutinating antibodies against SRBC's.

**Organs Measurements**

As shown in table (5) quail chicks exposed to Cd either at low or high level had significantly lower dressing and relative tibia weight as compared with those of control, phytase and 5 ppm Cd in combination with phytase. Opposite trend was noted for the relative weights of liver, kidney, heart, gizzard and brain.

**Table 5: Effect of phytase (Ph) supplementation on relative percentage weights of dressing and some organs of quail chicks during cadmium (Cd) intoxication.**

| Variable | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Control +Ph | 5ppm Cd | 50ppm Cd | 5ppm Cd+ Ph | 50ppm Cd+ Ph | MSE | Sig. |
| Dressing | 69.57 $^{a}$ | 70.32$^{a}$ | 66.81 $^{b}$ | 62.42 $^{c}$ | 69.22 $^{ab}$ | 63.47 $^{c}$ | 3.588 | *** |
| Liver | 2.33 $^{b}$ | 2.22 $^{b}$ | 2.26 $^{b}$ | 2.87 $^{a}$ | 2.42 $^{b}$ | 2.92 $^{a}$ | 0.053 | *** |
| Heart | 0.90 $^{bc}$ | 0.87 $^{c}$ | 0.97 $^{ab}$ | 1.06 $^{a}$ | 0.92 $^{bc}$ | 0.101 $^{ab}$ | 0.006 | *** |
| Kidney | 0.64 $^{d}$ | 0.70 $^{cd}$ | 0.80 $^{c}$ | 1.09 $^{a}$ | 0.74 $^{cd}$ | 0.97 $^{b}$ | 0.007 | *** |
| Gizzard | 2.58 $^{b}$ | 2.42 $^{b}$ | 2.43 $^{b}$ | 3.20$^{a}$ | 2.47 $^{b}$ | 3.02$^{a}$ | 0.058 | *** |
| Brain | 0.40 $^{d}$ | 0.43 $^{cd}$ | 0.42 $^{cd}$ | 0.57 $^{a}$ | 0.41 $^{cd}$ | 0.51 $^{b}$ | 0.002 | *** |
| Tibia | 0.54 $^{a}$ | 0.55 $^{a}$ | 0.47 $^{b}$ | 0.38 $^{d}$ | 0.54 $^{a}$ | 0.44 $^{c}$ | 0.005 | *** |

**$^{a-d}$Means within the same row with different superscripts are significantly different, ***P≤0.001.**

Our findings are in agreement with Akyolcu *et al*. (2003) and Herzig *et al*. (2007). An interpretation for the hypertrophy of internal body organs was introduced by Vodela *et al*. (1997) who showed incidence of necrotic lesions in gizzard, liver and kidney. An increase in heart weight usually develops as a compensatory response to an increase in work load (e.g., systemic hypertension) (Haschek and Rousseaux, 1991). Long-term exposure of laboratory animals to low levels of cadmium resulted in atherosclerosis and hypertension (Ramos *et al*., 1996).

## Histological Observation

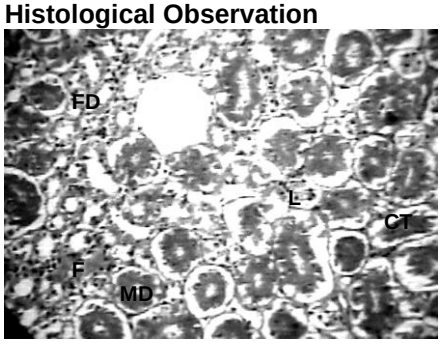


Plate (1) T.S. in the kidney of 50 ppm Cd + ph. Many medullary collecting ducts (MD) intermingling with cortical tubules (CT) along with segments of the fine collecting ducts (FD). Some lymphatic nodules (L) and fibroblasts (F) also seen.

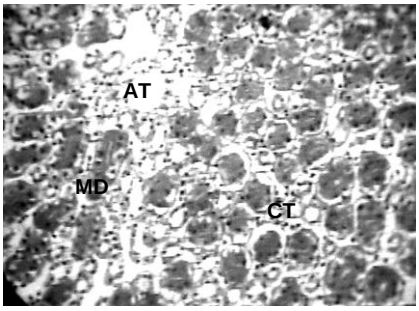


Plate (2): T.S. in the kidney of 5 ppm + ph. Normal appearance of renal tissues showing a pale medullary layer containing many dilated medullary ducts (MD) surrounded by cortical tubules (CT). The epithelial lining of these ducts are clear and darkly stained however, some atrophied tubules (AT) could be seen.

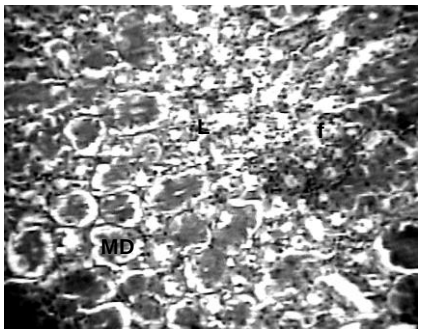


Plate (3).

Plate (4)

Plates (3 and 4) T.S. in kidney of 50 ppm Cd polluted chicks. There were more medullary ducts (MD) in the medullary layer but with a dark stain and disrupted epithelial linning. A fibrotic (f) areas with many lymphocytes (L) and fibroblasts cells in the cortical areas could be observed (plate, 3). Many congested blood vessels and metal precipitates are also present ( plate, 4).

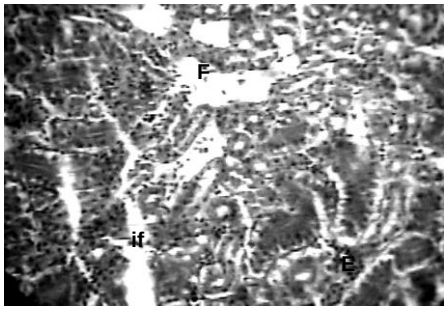


Plate (5). T.S. in the kidney of 5 ppm Cd exposed chicks. The diameter and number of the collecting ducts were larger than the other sections. Some fibrotic areas (F) are seen accompanied by infilterable fluid (if) and changes in the epithelial lining (E) of the duct.

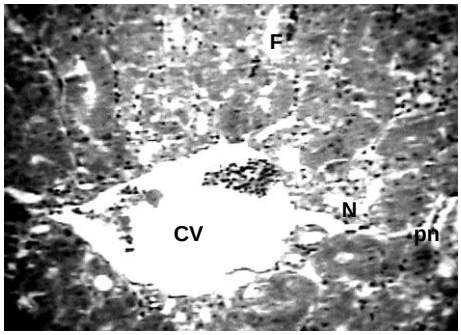


Plate 6) T.S in liver of 50ppm + ph. A marked disruption in the arrangement of hepatocytes mass and dilation of the central vein (CV) accompanied with many necrosis (N) and prencrotic areas (Pn). Three are many foci lesions (F) indicative of possible higher Cd concentration in liver tissues.

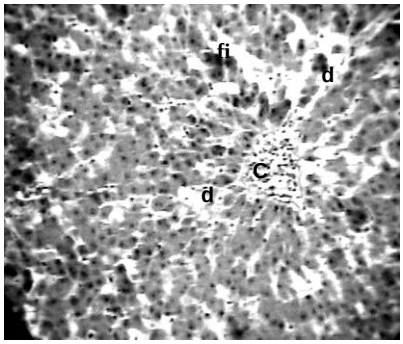


Plate (7): T.S. in liver of 5 ppm Cd+ph. Several types of degenerative changes (d) could be seen accompanied with congested (c) portal vein many fibrotic areas (fi). However, normal arranged of liver cells was noticed although the presence of some hyperplastic cells surrounding the portal vessel (arrows).

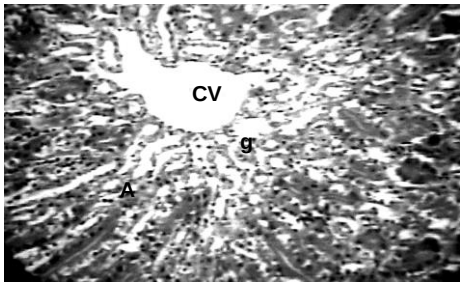


Plate (8)

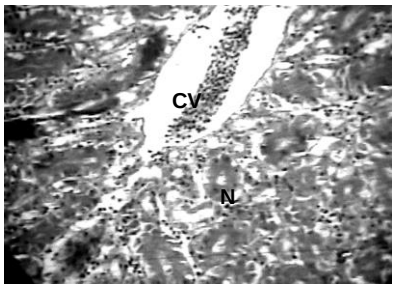


Plate (9)

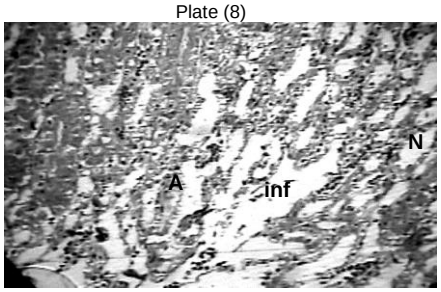


Plate (10)

(Plates 8, 9 and 10) T.S. in liver of 50 ppm Cd polluted chicks. A sever damage of hepatocytes including granular and vascular (g) degeneration of liver cells (Plate 8) accompanied with disorganization of the hepatic structure and infilterable fluid (inf) with many necrotic and congested areas (N) (plate 9, 10). Amorphous cells (A) including numerous nuclei were diagnostic for typical neoplastic areas.

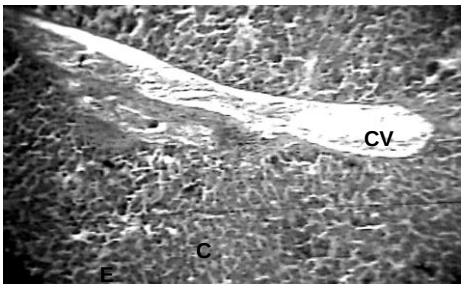


Plate (11). T.S. in liver of 5 ppm Cd exposed chicks. A sight vacuolar degeneration of liver cells was oobserved but sever dilation of the central vein (CV) with many congested areas (C) inbetween hepatocytes. The endothelial (E) linning of the vein was disrupted showing signs of fibrosis (F) near the vessel.

It could be concluded that phytase supplementation reduce the adverse effect of cadmium on growth performance, hematological and physiological aspects and immune response. However, more researches are needed to determine the optimal level of phytase under different levels of cadmium contamination to achieve normal productive and physiological status of Japanese quails.

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**إنزيم الفيتيز يقلل سمية الكادميوم فى السمان اليابانى**

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استخدم في هذه التجربة عدد ١٨٠ كتكوت سمان يابانى عمر ١٤ يوم وتم وزنها بصورة فردية وتوزيعه عشوائيا على ست معاملات لكل منها ثلاثة مكررات تضم عشرة طيور. استمرت التجربة حتى عمر ٤٢ يوم من العمر.تم إضافة عنصر الكادميوم فى صورة كلوريد كادميوم الى العليقة القاعدية بمستويات صفر, ٥ , ٥٠ مللجم/كجم مع أو بدون إنزيم الفيتيز بمستوى١٠٠٠وحدة.

وضحت النتائج أن مستوى ٥٠ مللجم كادميوم قد أعطى انخفاضا معنويا ملحوظا فى كل من وزن الجسم والأستهلاك الغذائى وكفاءة التحويل الغذائى بينما سجل مستوى ٥ مللجم كادميوم انخفاضا طفيفا للمقاييس السابقة مقارنا بمجموعة الكنترول. أدت أضافة انزيم الفيتيز الى المعالجة الكاملة أوالجزئية للتأثيرات السيئة على مقاييس النمو لكل من مستوى ٥ ,٥٠ مللجم كادميوم/كجم عليقة على الترتيب. أظهرت المجاميع المغذاة على علائق محتوية على عنصر الكادميوم انخفاضا معنويا خطيا فى كل من الهيماتوكريت والهيموجلوبين ومحتوى بلازما الدم لكل من البروتين الكلى ومشتقاته والكالسيوم والفوسفور بينما زاد على العكس من ذلك كل من الالبيومين الى الجلوبيولين وحمض اليوريك والكرياتنين وAST و ALT دليلا على انخفاض كفاءة الكبد والكلى بالمقارنة بالمجاميع المغذاة على عليقة الكنترول بدون او مع أضافة انزيم الفيتيز. كان لانزيم الفيتيز المقدرة على تلافى التأثير السيئ للكادميوم تبعا للجرعات المستخدمة. أظهرت الطيور التى تعرضت لمستويا ت ٥ ، ٥٠ مللجم كادميوم/كجم عليقة استجابة مناعية ذاتية منخفضة مقارنا بكلا من عليقتى الكنترول، بينما لم يكن هناك فروق معنوية بين المجموعة المغذاه على ٥ مللجم كادميوم مضافا لها انزيم الفيتيز وتلك الخالية من الكادميوم (control). لوحظ انخفاضا معنويا للوزن النسبى للذبيحة و عظمة الساق بينما لوحظت زيادة معنوية في الوزن النسبي لباقي الأعضاء (الكبد والكلى والقلب والقانصة والمخ) لطيور المعرضة للمستوى المنخفض او العالى من الكادميوم مقارنا بمجموعتى الكنترول أو٥ مللجم كادميوم مع الفيتيز. اظهر الفحص الهستولوجي حدوث درجات متفاوتة من التلف في أنسجة الكبد والكلية للسمان المعرض للكادميوم اعتمادا علي الجرعة الملوثة. بينما تحسن المظهر الهستولوجي لهذه الأنسجة مع إضافة الفيتيز إلي المستوي ٥ ملجم كادميوم/كجم.

نستخلص من هذه التجربة انه يمكن استخدام إنزيم الفيتيز لمعالجة أو تقليل التأثيرات السيئه لعنصر الكادميوم على الصفات الانتاجية والفسيولوجية والمناعية لكل من المستوى المنخفض والعالى المستخدم على الترتيب وان هناك حاجة لمزيد من الابحاث لدراسة تأثير استخدام مستويات مختلفة من إنزيم الفيتيز عند مستويات متعددة من التلوث بالكادميوم للوقوف علي أفضل النسب بينهما.

**2554 2555 2556 2557 2558 2559 2560 2561 2562 2563**

**2564 2565 2566 2567**