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Effects of Cadmium Administration on Reproductive Performance of Japanese Quail (*Coturnix japonica*)

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The effects of a single dosage of cadmium (Cd) administration on the survival rate, body weight (BW), egg production, egg quality characteristics and hatching outputs in 0, 0.1, 0.3, 1, 3 and 10 mg $CdCl_2$/kg BW injected laying quail were studied. It was found that a 10 mg Cd injection induced 41.7% mortality. A retainable significant BW loss in the 3 mg dosed birds and a decreased egg production in the 1 mg and 3 mg dosed birds were observed during the first 3 days after injection, and the recovering of the egg production rate was delayed as the doses of Cd injection increased. Cd administration significantly increased the shell membrane weight in 0.1 mg and 0.3 mg injection, decreased the eggshell thickness in 0.3 mg injection, and lowered the fertility rate in 0.3 mg injection during the first 3 days of injection. The present study suggests that exposure to Cd, even by a single dosage, can hamper quail reproduction for a very short time, mainly by decreasing egg production and thinning the eggshell.

Key words : cadmium, egg production, eggshell, Japanese quail, shell membrane

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Introduction

Numerous studies have shown that Cd and other heavy metal pollution influences the stability of populations by increasing mortality or decreasing reproductive output (reviewed in Peakall, 1992 ; Burger, 1995). Decrease in body weight (BW) in birds due to Cd exposure at sublethal levels has been reported by Sant'Ana *et al.* (2005). The number of eggs produced, an indicator of reproductive success in avian species, is also found to be lowered due to Cd and other heavy metals toxicity (Bokori *et al.,* 1995). Scheuhammer (1987) reviewed several effects of Cd pollution on avian reproduction, including male and female fertility. Cd and other metal pollutants have also been reported to affect generation continuity through decreasing fertility (Paksy *et al.,* 1996), embryonic death (Juhász *et al.,* 2005) and organ deformities in offspring (Baranski, *et al.,* 1985, 1986). However, the manifestation of these effects in exposed organisms is either the result of the unique action of Cd or the consequences of synergisms/antagonisms with other molecules (Mills and Delgarno, 1972).

Many researchers found that heavy metal pollution can cause thinning of the eggshell, which is among the first sign of the detrimental effects on the reproduction of birds (Eva and Lehikoinen, 1995). Thinner eggshells may reduce hatching success because eggs may break during incubation or dry out due to excessive evaporation (Drent and Woldendorp, 1989). Although many studies have been done to evaluate the impact of long-term Cd exposure in birds, the effects exerted by a single exposure of Cd

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administration on the overall reproductive performances of avian species has not been fully studied. The present study investigated the effects of Cd exposure by a single dose on BW, egg production, egg quality parameters and hatching outputs using a laboratory animal model, the Japanese quail.

Materials and Methods

Fifteen week-old laying Japanese quail (n=72) were used. Cd chloride ($CdCl_2 \cdot 2.5 H_2O$), purchased from Wako Pure Chemical (Tokyo, Japan) was the source of the Cd. The birds were reared in a light proof environmentally controlled house at 25℃ with a photoperiod of 14L : 10D. Two females were housed with one male in a $40 \times 15 \times 14\ cm^3$ battery cage pen. The birds were supplied with commercial laying quail diets and tap drinking water *ad libitum*. Five dosages of $CdCl_2$, 0.1, 0.3, 1, 3 and 10 mg/kg BW were used to inject the female birds intraperitoneally. The $CdCl_2$ was dissolved in saline and a volume of 0.3 m*l* contained the desired amount of $CdCl_2$ for a bird.

Every day between 9 : 30–10 : 30 am, the BW of each bird was measured individually. The number of eggs produced and dead birds (if any) under each dosage group were recorded every morning up to 14 days after injection. The collected eggs were weighed, and approximately half of the eggs laid by each dosage group were set in an incubator for hatching. The remaining half of the eggs were used to measure the yolk weight, shell membrane weight and eggshell thickness.

The yolks were carefully separated from the egg whites with forceps and blotting papers, and weighed. The shell membranes were completely removed from the eggshell using forceps after 30 minutes soaking in tap water. The membrane weight was taken after air drying in room temperature when the samples attained nearly constant weight. Eggshell thickness was measured with a digital micrometer (Mitsutoyo IP65, Japan) as reported (Ri *et al.,* 2005).

The eggs for hatching were set in an incubator at 39℃ and 65% humidity. On days 17 and 18 of incubation, successfully hatched-out chicks were weighed and the unhatched eggs opened to check for infertility and embryonic death (Tiwary and Maeda, 2005).

The recorded data were subjected to ANOVA followed by Duncan's multiple range test, except the data of egg production, fertility and hatchability, which were directly subjected to Fisher's exact probability test.

Results

It was found that the 10 mg Cd/kg BW injection induced 41.7% mortality of the birds within 24 hr after injection, while all birds survived in other dosages of Cd administration. Some of the surviving birds in the 10 mg dosed group showed symptoms like head jerking, curling of the head backward, and the laying of shell-less eggs. Fig. 1 showed a significant decrease in the BW of birds that received 3 mg Cd/kg BW during the first 3 days after injection. The results also showed that a Cd injection below a 3 mg/kg BW dosage caused an insignificant BW loss in the birds that was recovered within 4 days after injection, but the birds injected with 3 mg Cd/kg BW regained their BW slowly, approximately by 2 weeks after injection.

The percentage of hen-day egg production of the 3 mg Cd/kg BW injected birds were significantly lower than that of the control during 14 days after injection (Fig. 2). The 1 mg Cd/kg BW injection also significantly lowered the egg production rate further than that of the control during the first 4 days of injection.

The egg quality characteristics and hatching performances were examined only in the 0.1 mg and 0.3 mg Cd/kg BW administrated birds, because other

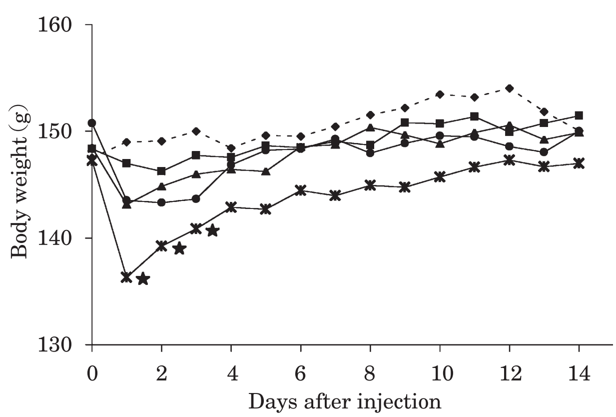

Fig. 1. **Body weight of laying Japanese quail after a single dosage of cadmium injection.** Dose of cadmium/kg body weight : ◆ 0 mg ; ■ 0.1 mg ; ▲ 0.3 mg ; ● 1 mg ; ✱ 3 mg. Each point indicates average of 12 birds. ★ $P<0.05$ when compared with the control.

groups produced a significantly lower number of eggs. Although neither the egg weight (Table 1) nor yolk weight (Table 2) were found to be markedly affected by Cd administration up to the end of the experimental period, the 0.3 mg Cd/kg BW injection to birds caused a significant decrease in eggshell thickness throughout the experimental period, while 0.1 mg Cd administration significantly decreased the eggshell thickness during 4–7 days of injection (Table 3). The shell membrane weights were found to be increased in both the 0.1 mg and 0.3 mg dosed birds up to 14 days after injection (Table 4).

A dose-dependent decrease in fertility rates were observed in the Cd injected laying quail during the first 3 days after injection (Table 5). No remarkable effects of either the dose of Cd or the days after injection on hatchability rate were found throughout the experimental period (Table 6), while a decreasing tendency in hatchling weight were observed in both the 0.1 mg and 0.3 mg dosed birds until the end of the experiment (Table 7).

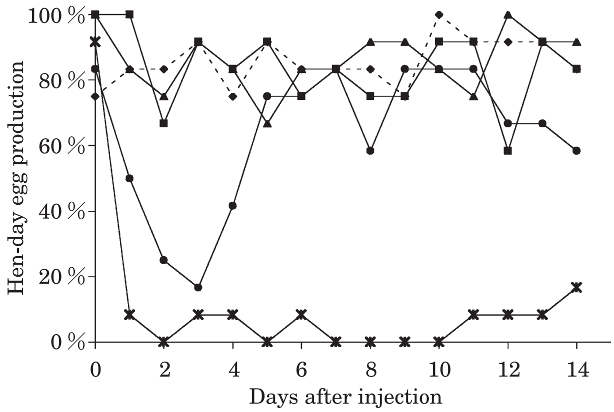

Fig. 2. **Hen-day egg production after single dosage of intraperitoneal cadmium injection in laying Japanese quail (n=12).** Dose of cadmium/kg body weight : ◆ 0 mg ; ■ 0.1 mg ; ▲ 0.3 mg ; ● 1 mg ; ✱ 3 mg. The data of 1–14 days of 3 mg and 1–4 days of 1 mg are significantly different from those of the corresponding days of the control ($P<0.05$).

Discussion

A mortality rate of 41.7% after the 10 mg Cd/kg BW injection indicated that the LD50 of Cd for quail is around this dosage. The BW loss of the Cd-injected laying quail found in our study might be the result of wide toxic effects by Cd on the whole body processes of the birds. In support of this assumption, it has been reported that long-term Cd exposure causes depletion of liver and muscular glycogen, due to its action on the enzymes involved with glycogenesis and energy metabolism (Tourry *et al.,* 1985) and induces oxidative stress in the liver and kidney (Yiin *et al.,* 1999). Studies have shown that Cd causes progression of diabetic renal complications, hypertension, osteoporosis and leukemia in living animals and humans (reviewed by Jarup *et al.,* 1998). Thus, these cumulative effects might result in BW loss of Cd exposed animals.

Table 1. **Effects of intraperitoneal cadmium injection on egg weight in Japanese quail**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	10.6±0.53 (30)	10.9±0.44 (40)	10.8±0.56 (74)
0.1	10.4±0.93 (31)	10.5±0.67 (40)	10.7±0.75 (68)
0.3	10.3±1.01 (30)	10.6±0.89 (38)	10.6±0.82 (75)

Values (g) are mean±SD of the number of eggs in parenthesis.

Table 2. **Effects of intraperitoneal cadmium injection on egg yolk weight in Japanese quail**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	28.9±1.41 (15)	29.5±2.02 (19)	30.0±1.55 (38)
0.1	29.1±1.94 (15)	29.9±2.21 (20)	29.6±2.20 (33)
0.3	28.6±1.26 (16)	29.3±2.75 (19)	29.5±2.10 (35)

Values (% to egg weight) are mean±SD of the number of eggs in parenthesis.

Table 3. **Effects of intraperitoneal cadmium injection on eggshell thickness in Japanese quail**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	0.220±0.018 (15)	0.213±0.008 (19)	0.212±0.011 (38)
0.1	0.215±0.025 (15)	0.203±0.014* (20)	0.211±0.015 (33)
0.3	0.208±0.013* (16)	0.206±0.018 (19)	0.206±0.012* (35)

Values (mm) are mean±SD of the number of eggs in parentheses.
* $P < 0.05$, when compared with the control in the same column.

Table 4. **Effects of intraperitoneal cadmium injection on shell membrane weight in Japanese quail**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	0.41±0.07 (15)	0.41±0.06 (19)	0.41±0.07 (38)
0.1	0.48±0.13 (15)	0.47±0.10* (20)	0.46±0.08* (33)
0.3	0.50±0.06* (16)	0.47±0.06* (19)	0.46±0.08* (35)

Values (% to egg weight) are mean±SD of the number of eggs in parentheses.
* $P < 0.05$, when compared with the control in the same column.

Table 5. **Fertility rate of eggs laid by Japanese quail receiving intraperitoneal cadmium injection**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	100 (15)	90.5 (21)	97.2 (36)
0.1	81.3 (16)	95.0 (20)	85.7 (35)
0.3	71.4* (14)	100 (19)	97.5 (40)

Values (%) are mean of the number of eggs in parentheses.
* $P < 0.05$, when compared with the control in the same column.

Table 6. **Hatchability of fertilized eggs laid by Japanese quail receiving intraperitoneal cadmium injection**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	100 (15)	84.2 (19)	100 (35)
0.1	92.3 (13)	89.5 (19)	90.0 (30)
0.3	90.0 (10)	100 (19)	97.4 (39)

Values (%) are mean of the number of fertile eggs in parentheses.

Table 7. **Weight of hatchling from eggs laid by Japanese quail receiving intraperitoneal cadmium injection**

Dosage (mg/kg BW)	Days after injection		
	1–3	4–7	8–14
0	70.5±3.2 (15)	70.5±2.0 (16)	68.9±3.5 (35)
0.1	69.9±5.1 (12)	69.6±3.5 (17)	69.0±3.6 (27)
0.3	68.6±2.8* (10)	68.0±3.5 (19)	69.9±3.8 (38)

Values (% to egg weight) are mean±SD of the number of chicks in parentheses.
* $P < 0.05$, when compared with the control in the same column.

Eggs are both a reproductive unit and one of the excretory routes in female birds (Burger, 1994). The significant decrease of egg production in the Cd-injected quail found in the present study could have resulted from alterations in the egg formation pathway. Our result was in agreement with those of some researchers who showed reduced egg production in quail (Bokori *et al.,* 1995) and in chickens (Vodela *et al.,* 1997). The dose and period of Cd exposure may be an important factor altering egg production potentialities which could be confirmed by the findings that showed no effect of Cd contamination on the egg production of pheasants given drinking water containing 1.5 mg Cd/L (Toman *et al.,* 2005).

The eggshell is mainly composed of calcium-bicarbonate and is formed by the biochemical reaction of calcium, CO_2, and H_2O in the presence of carbonic anhydrase in the shell gland of the female reproductive tract in birds (Roberts, 2004). Chemical analysis has also shown the presence of other minerals in eggshells (Dauwe *et al.,* 2005). From the eggshell composition and formation procedure, it could be hypothesized that poorer formation or thinness in eggshells may be the result of the unavailability or imbalance of the component matters needed for the chemical reaction in eggshell formation. The thinner eggshells and some shell-less eggs found in our study could strengthen this hypothesis. Furthermore, Cd was reported as antagonistic for divalent cations like calcium, zinc, copper, iron, and these are needed for eggshell formation (Bokori *et al.,* 1995). Our findings were in agreement with the results of some researchers (Eeva and Lehikoinen, 1995 ; Toman *et al.,* 2005) who reported a thinner eggshell in pheasants (*Phasianus colchicus*), pied flycatchers (*Ficedula hypoleuca*) and great tits (*Parus major*) exposed to environmentally elevated concentrations of heavy metals.

The dose-dependent increase of the shell membrane weight observed in the Cd-injected birds might compensate for the thin eggshell disability found in the present study. The increase of the shell membrane weight would minimize evaporation and lead to better hatchability. However, no report is available on the mechanism of Cd-mediated increase of the shell membrane weight. The increased weight of the shell membrane in Cd-injected quail might be the result of the positive influence of Cd on the secretory mechanisms of membrane proteins and glycogens in the oviduct mucosa, which have not been clearly understood until recently (Ohashi *et al.,* 2003).

Our study showed no effects of Cd administration on hatchability that could be explained by the lower level of Cd transferred into eggs to affect the hatching success. This idea coincided with the reports of some researchers who found Cd to be restrictedly transferred into the egg yolks of Cd-exposed hens (Sato *et al.,* 1997). The satisfactory level of hatchability in Cd-administered hen eggs as well as in eggs directly injected with 1.2 μg Cd/egg was also noted (Sato *et al.,* 1997). The decreased hatchling weight found in Cd-injected quail might be the result of Cd toxicity in developing embryo. A significant decrease in the live weight of newborn rabbits after dosing their mother with 1.0 mg Cd/kg feed for 5 months was in agreement with our present findings (Massanyi *et al.,* 1995). However, the increased hatchling weight of pheasant fed 1.5 mg Cd/L via drinking water only after 4 weeks and not before 3 weeks of administration (Toman *et al.,* 2005) contradicted our result indicating the importance of the length of Cd exposure on hatchling weight.

The regaining of BW and egg production within the first few days after Cd administration indicated the detoxifying potentiality of Cd toxicity, which already has been proven in mammals (Xu *et al.,* 2005). Finally, it was concluded that a single dose of Cd administration can decrease the egg production and BW of laying quail in a dose-dependent manner which was quickly recoverable. In addition, a long period of eggshell thinning was the pronounced impact of Cd toxicity in laying quail.

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