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# Effect of Dietary Lead on Reproductive Performance in Japanese Quail, *Coturnix coturnix japonica*[1,2]

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Effect of Dietary Lead on Reproductive Performance in Japanese Quail (*Coturnix coturnix japonica*). EDENS, F. W., BENTON, E., BURSIAN, S. J., AND MORGAN, G. W. (1976). *Toxicol. Appl. Pharmacol.* **38**, 307–314. Japanese quail received dietary lead at levels ranging from 1 to 1000 μg/g of feed from hatching through 12 weeks of age. Levels of lead which did not cause depressed growth rates, anemia, or any other overt signs of chronic plumbism caused a decrease in reproductive capacity in the females. This decrease in reproductive capacity appeared to be related to impaired calcium metabolism.

The pathological manifestations of plumbism in mammals have traditionally involved the central nervous system, the hematopoietic system, and the renal system. However, plumbism in birds has not been studied intensively and only recently have reports indicated that lead may affect birds in much the same way as it does mammals (Vengris and Moré, 1974; Morgan *et al.*, 1975). Lead toxicity studies in wildfowl (Bagley and Locke, 1967; Benson *et al.*, 1974; Haegele *et al.*, 1974) have primarily involved determinations of the distribution of lead in body tissues and the correlation of these concentrations with mortality. Karstad (1971) and Sileo *et al.* (1973) have shown that lead in waterfowl produced severe myopathies which caused characteristic changes in electrocardiograms. Irwin and Karstad (1972) reported that metallic lead in ducks caused emaciation, proventricular impaction, gizzard erosion, liver atrophy, gall bladder distention, hydropericardium, and degeneration of kidney tubules. Stone and Soars (1974) indicated that Japanese quail exposed to lead produced significantly more soft shell eggs than control birds. Salisbury *et al.* (1958) reported that lead intoxication in adult hens caused a significant weight loss which was accompanied by cessation of egg production, severe anemia, and high mortality.

Recently, we reported on the development of the signs of lead toxicity from hatching through 6 weeks of age in the Japanese quail (Morgan *et al.*, 1975). Some of the quail

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9217

involved in those experiments were maintained and used in pilot experiments to ascertain the feasibility of studying reproductive traits in lead-exposed birds. The results suggested that lead may have had a deleterious effect on reproduction in the Japanese quail. In our initial investigation (Morgan *et al.*, 1975), we proposed that the Japanese quail be considered as a model for future investigations of plumbism. The intent of the current study is to provide support for this proposal, and the specific objective is to determine the effect of dietary lead on reproductive traits in the Japanese quail.

## METHODS

Unsexed Japanese quail chicks from the North Carolina Agricultural Experiment Station flock were used in each of two replicate experiments. The duration of each experiment was 12 weeks and the experimental designs were identical. The chicks were colony-reared in metal brooding batteries from hatching until 6 weeks of age. The brooding temperature was maintained at 35°C during the first week, at 30°C during the second week, and at 25°C during the next 4 weeks. The experimental diets during the first 6 weeks consisted of a nutritionally complete quail starter-grower ration to which lead in the form of lead acetate was added at the levels of 0, 1, 10, 100, or 1000 $\mu$g/g of feed. In all cases the amount of lead was corrected for the acetate moiety and the levels are expressed as parts per million (ppm). When the quail were 6 weeks old, they were provided with a layer ration to which the same concentrations of lead were added. The experimental diets and water were available *ad libitum*. At 6 weeks of age, 20 males and 20 females from each of the five experimental groups were housed as pairs in quail laying cages. Thus, each of the 20 × 18 × 19-cm cages contained one male and one female which had been reared on the same level of lead.

The chicks were on a continuous 24-hr light schedule for the first week. When the quail were 1 week old, the lighting was reduced to 10 hr of light and 14 hr of dark (10L:14D) per day for 1 week. Thereafter the lighting was increased by 1 hr per week until a 14L:10D schedule was reached at 6 weeks. The quail remained on the 14L:10D schedule until they were sacrificed at 12 weeks of age.

Body weights were determined at 2, 4, 6, and 12 weeks of age. At 6 and 12 weeks of age, the quail were bled via the wing veins. The blood was collected from each bird in 0.25% heparin and centrifuged at 1000 *g* for 30 min. The resulting plasma samples were stored at −20°C for subsequent analysis of total calcium (Baginski *et al.*, 1974) and inorganic phosphate (Goldenberg and Fernandez, 1966). At 12 weeks of age, the quail were killed by cervical dislocation, and the relative weights of the liver, brain, testes, ovary, and oviduct were determined.

During the 6 weeks that the quail were mated in laying cages, records of egg production and weekly hatchability of setable eggs were maintained. In addition, the age at sexual maturity of the females, i.e., the age when the first egg was laid, was determined. Analyses of variance were performed on all data according to Ostle (1963) and differences among treatment means were determined by Kramer's (1956) modification of the Duncan multiple range test. Statements of significance are based on differences at the 5% level of probability. Since similar trends were seen in the data from the two replicate experiments, data for the individual parameters were analyzed and are presented collectively for this report.

## RESULTS

### *Inhibition of Growth by Dietary Lead*

The effects of dietary lead on the growth of the quail are presented graphically in Fig. 1. The addition of 1, 10, or 100 ppm of lead to the diet had no significant effect on growth during the experimental period. However, dietary lead at the level of 1000 ppm

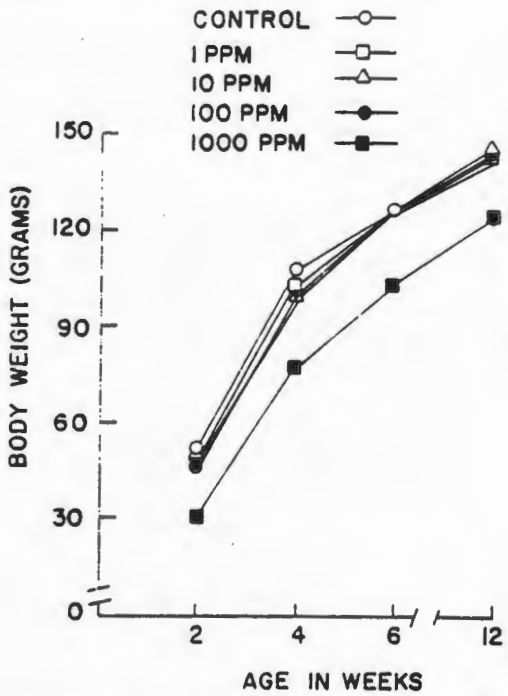


FIG. 1. Body weights of Japanese quail given dietary lead from hatching through 12 weeks of age. Equal numbers of both male and female quail are represented in each mean within a treatment.

significantly inhibited growth. Through 6 weeks of age the depressed body weights seen in the 1000-ppm group were evident in both the males and females. However, at 12 weeks of age only the body weights of the females were affected significantly.

### *Lead-Induced Reproductive Dysfunction in Females*

The effect of lead on egg production is presented in Fig. 2. As little as 1 ppm of lead added to the diet caused a significant depression in egg production. Higher levels of lead resulted in more pronounced depressions in egg production. Dietary lead at the level of 1000 ppm almost completely suppressed egg production, and those eggs produced by the females receiving 1000 ppm of lead were usually soft-shelled or shell-less. The maximum rate of egg production occurred during the eighth week of age in the control and 1-ppm groups. The maximum rate of egg production in the groups fed 10 and 100 ppm was delayed at least until the twelfth week of age. However, egg production in all lead-treated groups was lower than in the controls.

The age at sexual maturity was significantly increased by dietary lead at the levels of 10, 100, and 1000 ppm. In addition, treatment with 1000 ppm of lead significantly increased age at sexual maturity when compared to the 10- and 100-ppm lead groups

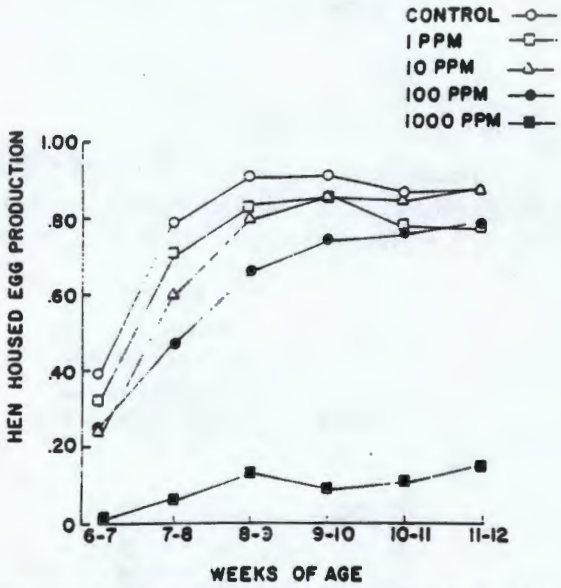


FIG. 2. Hen-housed egg production (percentage of hens producing eggs in a given time period) in Japanese quail hens given dietary lead as lead acetate from hatching through 12 weeks of age.

TABLE 1

AGE OF SEXUAL MATURITY, SETABLE EGGS AND HATCHABILITY OF SETABLE EGGS FROM JAPANESE QUAIL GIVEN DIETARY LEAD FROM HATCHING THROUGH 12 WEEKS OF AGE

| Treatment (ppm) | Days of age at first egg | Setable eggs/hen/week | Percentage hatch of setable eggs |
|---|---|---|---|
| Control | $46.2 \pm 0.8^{a}$ | $6.0 \pm 0.2^{a}$ | $81.6 \pm 2.1^{a}$ |
| 1 | $47.4 \pm 0.9^{a,b}$ | $5.4 \pm 0.2^{b}$ | $75.2 \pm 7.0^{a}$ |
| 10 | $49.7 \pm 0.8^{b}$ | $5.2 \pm 0.1^{b}$ | $82.4 \pm 1.6^{a}$ |
| 100 | $51.9 \pm 1.0^{b}$ | $4.2 \pm 0.3^{c}$ | $59.1 \pm 4.3^{b}$ |
| 1000 | $60.7 \pm 2.2^{c}$ | $0.3 \pm 0.1^{d}$ | $4.0 \pm 2.5^{c}$ |

$^{a-d}$ Means ± SE in a column with different superscripts are significantly different ($p < 0.05$) from each other.

(Table 1). Only 25% of the females fed 1000 ppm of lead ever produced eggs, while all of the females on the control and lower lead-level diets laid eggs. All levels of dietary lead caused a significant reduction in the number of setable eggs produced per hen per week (Table 1), and as dietary lead content increased there was a consistently greater depression in the number of setable eggs produced by the hens. The percentage hatch of setable eggs was significantly reduced in the group fed 100 ppm of lead and was further reduced by 1000 ppm of lead (Table 1).

*Plasma Calcium and Phosphate Response to Lead Exposure*

The levels of dietary lead used in these experiments did not cause significant changes to occur in the concentration of plasma calcium and inorganic phosphate in the males (Table 2). There was no change in plasma calcium from 6 to 12 weeks of age, but the

TABLE 2

PLASMA Ca AND $PO_4$ IN 6- AND 12-WEEK-OLD MALE JAPANESE QUAIL GIVEN DIETARY LEAD FROM HATCHING

| Treatment (ppm) | 6 Week Ca (mg/100 ml) | 6 Week $PO_4$ (mg/100 ml) | 12 Week Ca (mg/100 ml) | 12 Week $PO_4$ (mg/100 ml) |
|---|---|---|---|---|
| Control | 9.6 ± 0.5[a] | 5.9 ± 0.5 | 10.0 ± 0.7 | 3.1 ± 0.5 |
| 1 | 11.9 ± 0.7 | 6.2 ± 0.5 | 11.3 ± 0.9 | 4.0 ± 0.3 |
| 10 | 10.4 ± 0.8 | 5.1 ± 0.5 | 13.9 ± 1.3 | 3.8 ± 0.4 |
| 100 | 10.4 ± 0.3 | 6.1 ± 0.5 | 9.4 ± 1.0 | 3.7 ± 0.3 |
| 1000 | 10.0 ± 0.6 | 7.8 ± 0.7 | 10.0 ± 1.2 | 3.8 ± 0.3 |

[a] Means ± SE in a column are not significantly different from each other.

inorganic phosphate concentration in the 12-week-old males was only half that found in the 6-week-old males (Table 2). This reduction in inorganic phosphate was not lead-associated and probably reflected the lowered phosphate level in the diets of the 12-week-old birds.

In the 6-week-old females, dietary lead at the level of 10 ppm or greater caused a significant reduction in plasma total calcium (Table 3), and in the 12-week-old females

TABLE 3

PLASMA Ca AND $PO_4$ IN 6- AND 12-WEEK-OLD FEMALE JAPANESE QUAIL GIVEN DIETARY LEAD ACETATE FROM HATCHING

| Treatment (ppm) | 6 Week Ca (mg/100 ml) | 6 Week $PO_4$ (mg/100 ml) | 12 Week Ca (mg/100 ml) | 12 Week $PO_4$ (mg/100 ml) |
|---|---|---|---|---|
| Control | 15.5 ± 2.4[a] | 6.4 ± 0.6 | 46.9 ± 2.3[a] | 5.6 ± 0.5 |
| 1 | 13.5 ± 1.4[a,b] | 6.9 ± 1.0 | 36.5 ± 1.9[b] | 4.8 ± 0.8 |
| 10 | 11.1 ± 0.9[b] | 5.6 ± 0.5 | 38.6 ± 2.4[b] | 5.1 ± 0.4 |
| 100 | 12.1 ± 0.8[b] | 6.3 ± 0.5 | 36.0 ± 2.3[b] | 5.4 ± 0.5 |
| 1000 | 11.6 ± 1.0[b] | 8.0 ± 1.5 | 20.3 ± 2.6[c] | 5.0 ± 0.6 |

[a–c] Means ± SE in a column with different superscripts are significantly different ($p < 0.05$) from each other.

as little as 1ppm of dietary lead caused a significant reduction in plasma calcium. Plasma calcium concentrations were not different among the 12-week-old females receiving 1, 10, or 100 ppm of lead, but calcium concentration in the birds in the 1000-ppm group were significantly reduced compared to all other dietary levels of lead (Table 3).

The plasma inorganic phosphate concentrations in the females were not affected by dietary lead treatment at either 6 or 12 weeks of age (Table 3). There was a reduction in

the inorganic phosphate of the 12-week-old females compared to the 6-week females, but this reduction was not associated with lead treatment. This reduction may have been an expression of reduced inorganic phoshate in the diet of the 12-week-old birds.

*Reduced Organ Weights in Lead-Exposed Quail*

Body weights and relative weights of the testes, liver, and brain of 12-week-old males are presented in Table 4. There were no significant differences among treatment groups

TABLE 4

BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS OF 12-WEEK-OLD MALE JAPANESE QUAIL GIVEN DIETARY LEAD ACETATE FROM HATCHING

| Treatment (ppm) | Body weight (g) | Testes (mg/100 g) | Liver (g/100 g) | Brain (mg/100 g) |
|---|---|---|---|---|
| Control | 126 ± 3 | 2823 ± 69[a] | 2.04 ± 0.08[a] | 616 ± 20 |
| 1 | 127 ± 3 | 2447 ± 122[b] | 1.77 ± 0.07[b] | 614 ± 20 |
| 10 | 132 ± 4 | 2743 ± 99[a] | 2.02 ± 0.12[a] | 562 ± 27 |
| 100 | 133 ± 6 | 2774 ± 94[a] | 1.71 ± 0.05[b] | 574 ± 26 |
| 1000 | 124 ± 5 | 2441 ± 137[b] | 2.02 ± 0.09[a] | 611 ± 28 |

[a–b] Means ± SE in a column with different superscripts are significantly different ($p < 0.05$) from each other.

for body weight or relative brain weight. There was a general trend of reduced testicular weights in the birds of the lead-treated groups but only those groups fed 1 and 1000 ppm had significantly reduced testicular weights. The relative liver weights of the males in the 1- and 100-ppm lead-treatment groups were significantly reduced compared to controls and other lead-treatment groups.

The body weights and relative weights of the ovaries with maturing ova attached, oviducts, livers, and brains of 12-week-old females are presented in Table 5. There were

TABLE 5

BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS OF 12-WEEK-OLD FEMALE JAPANESE QUAIL GIVEN DIETARY LEAD ACETATE FROM HATCHING

| Treatment (ppm) | Body weight (g) | Ovary (g/100 g) | Oviduct (g/100 g) | Liver (g/100 g) | Brain (mg/100 g) |
|---|---|---|---|---|---|
| Control | 163 ± 4[a] | 3.41 ± 0.15[a] | 3.80 ± 0.10[a] | 3.50 ± 0.17[a] | 469 ± 15 |
| 1 | 159 ± 3[a] | 3.49 ± 0.19[a] | 3.71 ± 0.13[a] | 3.13 ± 0.11[a] | 458 ± 13 |
| 10 | 161 ± 4[a] | 3.71 ± 0.20[a] | 3.79 ± 0.17[a] | 3.39 ± 0.12[a] | 448 ± 12 |
| 100 | 157 ± 4[a] | 3.52 ± 0.19[a] | 3.70 ± 0.17[a] | 3.16 ± 0.09[a] | 467 ± 12 |
| 1000 | 129 ± 9[b] | 1.24 ± 0.19[b] | 1.36 ± 0.36[b] | 2.13 ± 0.13[b] | 479 ± 24 |

[a–b] Means ± SE in a column with different superscripts are significantly different ($p < 0.05$) from each other.

no significant differences among mean body weights of control females and those of 1-, 10-, and 100-ppm groups. However, females receiving 1000 ppm of dietary lead were found to have significantly reduced body weights. Relative brain weights were not

affected by dietary lead, but dietary lead at the level of 1000 ppm caused a significant reduction in relative weights of ovaries, oviducts, and livers.

## DISCUSSION

The relationship between chronic lead ingestion and development of pathophysiological manifestations has been suspected since the time of the ancient Greek empire. One of the primary signals of plumbism in mammals is growth inhibition (Grant *et al.*, 1938; Laug and Morris, 1938). Growth inhibition was reported as a sign of lead toxicity in Japanese quail by Morgan *et al.* (1975), and the results of the present study confirm and extend the earlier report. In the current experiment, growth inhibition was evident in both males and females fed high levels of lead through 6 weeks of age, but after 12 weeks of high levels of lead consumption only the females continued to show reduced body weights. Since female Japanese quail normally increase body weight by 20 to 40% during sexual maturation, this smaller body weight in the 12-week-old females fed the high levels of lead was attributed to the delayed sexual development as indicated by reduced ovarian weights.

The chronic exposure of mine workers to lead was believed also to be responsible for lowered fertility rates in their familes, but until Cole and Bachhuber (1914) reported reduced reproductive ability in lead-treated rabbits and roosters, no definitive correlation between lead and reproduction could be found. Today there appears to be a consensus that chronic exposure to high levels of lead will cause reproductive dysfunction in mammals (Schroeder and Michener, 1971; Stowe and Goyer, 1971; Hilderbrand *et al.*, 1973). Reproductive dysfunction is found in both sexes, but Stowe and Goyer (1971) and Hilderbrand *et al.* (1973) reported that female rats are more susceptible to lead exposure than males.

Morgan *et al.* (1975) reported that testicular development in the 6-week-old juvenile *Coturnix* quail was delayed when the birds consumed high levels of dietary lead. In the present study, at 12 weeks of age the relative testes weights of lead-exposed quail were lower in birds consuming 1 and 1000 ppm of dietary lead. Nevertheless, the testicular weights of all groups of quail in the present study were within the normal limits reported by Wilson *et al.* (1962) for mature Japanese quail. Since reproductive performance of the adult male was not evaluated in the present study, the possibility that sperm production and viability were impaired by chronic exposure to dietary lead is not precluded.

Reproductive dysfunction was apparent in the female quail receiving as little as 1 ppm of dietary lead, and higher levels of dietary lead further potentiated the response. This reproductive dysfunction may be associated with impaired calcium metabolism. Van Tienhoven (1968) states that normal reproductive capacity of laying hens is suppressed if calcium metabolism is impaired, and in this study chronic exposure to all levels of dietary lead caused a decrease in plasma calcium concentrations.

This is the first report of lead-induced reproductive dysfunction in the Japanese quail. The findings of this study are particularly striking since chronic exposure to levels of dietary lead that are conceivable in the environment, i.e., 1–25 ppm, caused a suppression in the reproductive capacity of females. Since the results of this study indicate that environmental levels of lead can affect reproduction in the Japanese quail, possibly through impaired calcium metabolism, and since lead affects other physiological

systems in the quail in the same way as it does in mammals, it is felt that the quail may be a new model for studying plumbism.

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