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Infuence of Dietary Calcium, Selenium, and Methylmercury on Eggshell Thickness in Japanese Quail

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Interest in environmental contaminants that have been associated with eggshell-thinning in wild birds, and in basic aspects of calcification and calcium metabolism has prompted studies on chemical interactions with eggshell formation (COOKE 1973; MUELLER and LEACH 1974). However, results of these studies are sometimes conflicting and contradictory, particularly for mercurial compounds (FIMREITE 1971; STOEWSAND et al. 1971; HILL and SHAFFNER 1976).

Selenium (Se) protection against toxicosis by mercury and other heavy metals is well documented (PARIZEK and OSTADALOVA 1967; FROST and LISH 1975). This protection probably results from selenium's strong complexing or binding tendency with metals (GANTHER et al. 1973). In confirming this phenomenon with both natural and inorganic Se, we have observed that selenite-Se added to Japanese quail (*Coturnix coturnix japonica*) diets containing methylmercury chloride (MHg) lowered both egg production and eggshell thickness (STOEWSAND et al. 1977). The present study was designed to see if the level of dietary calcium (Ca) would influence this MHg-Se effect on egg production, shell thickness, and Se-tissue residues in Japanese quail hens. In addition, estimates of the blood and oviduct carbonic anhydrase (CA) were determined since this enzyme catalyzes the formation of the carbonate radical of Ca carbonate that accounts for about 80% of the thickness of avian eggshell (MUELLER and LEACH 1974).

EXPERIMENTAL

Day-old Japanese quail were placed in an electrically heated commercial chick brood unit (light regimen, 16 L:8 D) with free access to a low Ca (1.3% Ca; 0.8% P) semi-purified soy protein-cornstarch diet previously described (STOEWSAND and ROBINSON 1970) and distilled water. At the end of 2 weeks, one half of the quail were placed on a high Ca (3.4% Ca; 1.3% P) diet. The low and high Ca groups were further divided whereby either 7 ppm Se ($NaSeO_3 \cdot 5\ H_2O$), or 7 ppm Se + 20 ppm MHg were added to each of the 2 Ca diets. Thus, there

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were 6 dietary treatments, low or high Ca, with or without added Se, or added Se + MHg. A MHg treatment without Se was not included since we have previously observed high mortality and no egg production in quail fed 20 ppm of MHg (STOEWSAND et al. 1977). There were 18-22 unsexed quail within each treatment. The experiment was terminated at 16 weeks or the end of the 10th week of laying.

During the 5th, 7th, and 10th week of laying, egg production was calculated as a % of eggs produced from the number of hens within each group. Broken and shell-less eggs were estimated. Eggshell thickness was measured on 15 eggs per treatment (10 only available in the 2 MHg groups) as previously described (STOEWSAND et al. 1977). One of us (JLA) did the measurements "blind", i.e. without knowledge of treatments.

CA (E.C. 4.2.1.1) activity was estimated on whole blood and oviduct of the quail hens (WILBUR and ANDERSON 1948). Se residues in the hens liver, kidney, and brain were determined (OLSON 1969).

RESULTS

Figure 1 shows egg production and the number of broken or shell-less eggs produced during the 5th, 7th and 10th weeks of their egg-laying period. Analysis of variance of the data shows that low dietary Ca depressed egg production significantly over these 3 weekly periods ($P<0.05$), but the depression was more pronounced ($P<0.001$) with the added Se + MHg. More broken or shell-less eggs were produced by hens fed low Ca diets than those on high Ca. About 70% of all eggs produced on the low Ca + Se-MHg diet were broken or shell-less.

Figure 2 indicates that 7 ppm of dietary Se reduced egg weights significantly ($P<0.005$); no further reduction occurred when MHg was added to the diets. The highly significant ($P<0.005$) positive influence of Ca on eggshell thickness appeared to be negated ($P<0.001$) with MHg treatment. Se fed alone did not reduce eggshell thickness. Quail hens fed the Se + MHg diets showed approximately a 20% reduction in shell thickness as compared to the eggs of the birds fed the optimal high Ca diet.

Table 1 shows Se residues in liver, kidney, and brain of the quail hens, and the estimates of CA activities in blood and oviduct. Feeding MHg increased the level of Se in these tissues ($P<0.05$), as compared to diets containing only Se. CA activity in blood of hens fed MHg + Se was significantly ($P<0.05$) higher

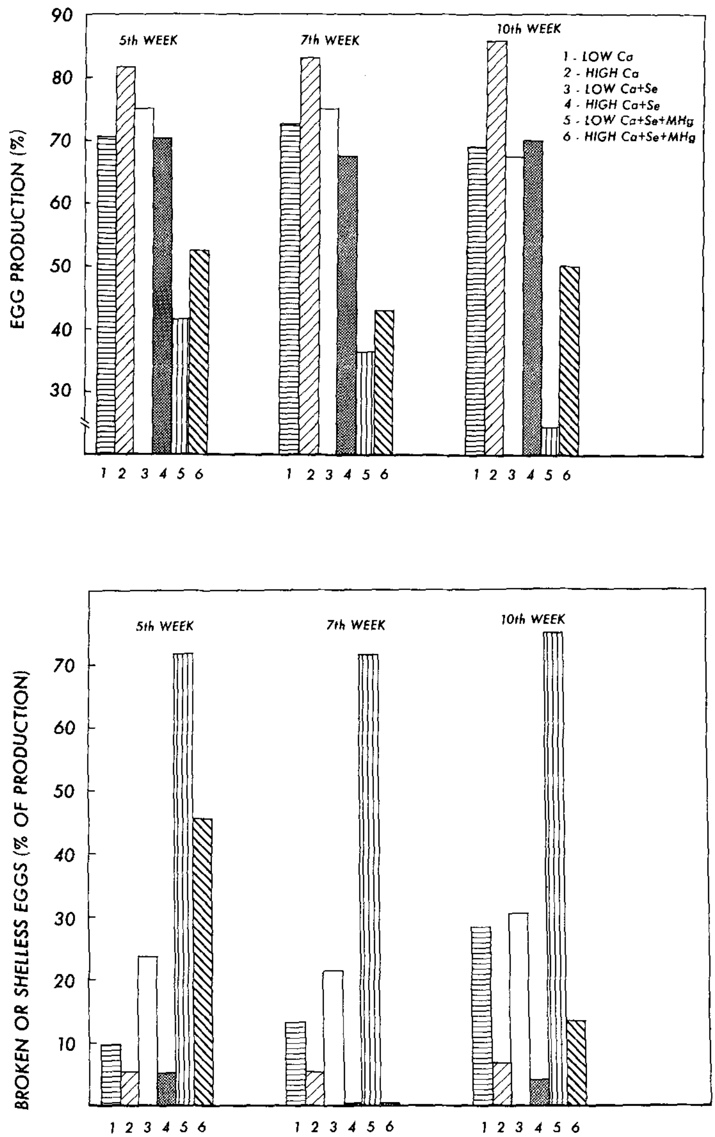

Fig. 1. Mean egg production (upper) and broken or shell-less eggs (lower) of Japanese quail hens fed 2 levels of dietary Ca with 7 ppm Se and 20 ppm MHg. Analysis of variance shows that Ca had a significant effect ($P<0.05$) on these parameters. Se-MHg diet reduced egg production and increased broken or shell-less eggs ($P<0.005$), especially in the low Ca treatment (group 5).

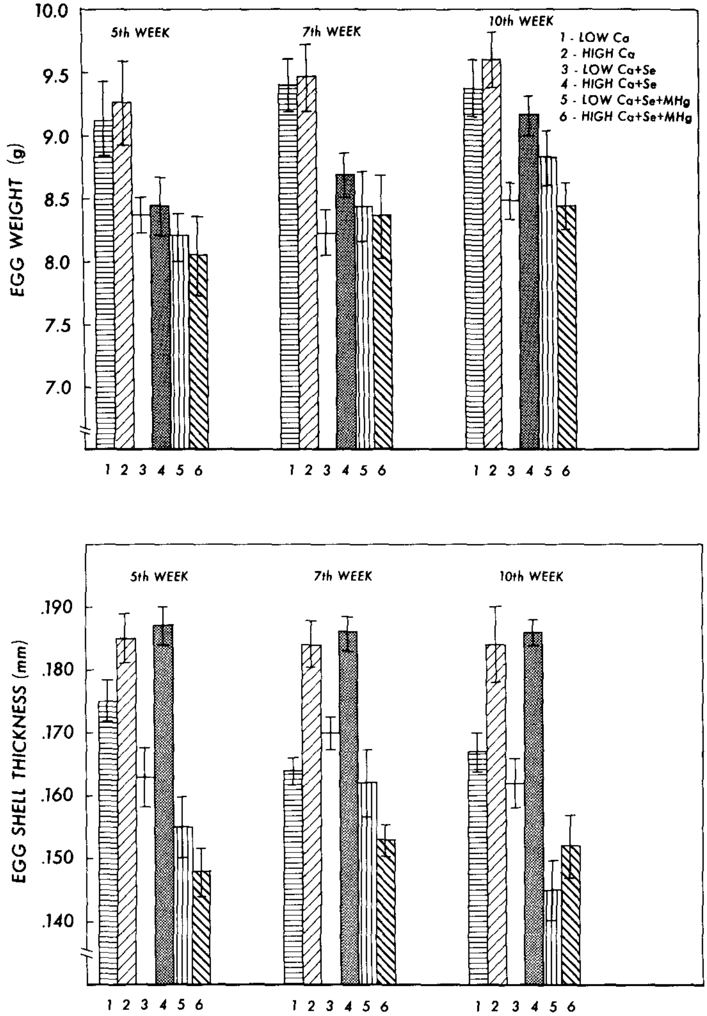

Fig. 2. Mean egg weight (upper) and eggshell thickness (lower) ± S.E. of quail fed 2 levels of dietary Ca with 7 ppm Se and 20 ppm MHg. Analysis of variance shows Ca did not have a significant effect on egg weights ($P>0.05$). Se depressed egg weights significantly ($P<0.01$) within each of the 3 weekly periods. Ca significantly increased eggshell thickness ($P<0.01$), with or without the addition of Se. MHg depressed eggshell thickness ($P<0.005$) and negated the normally increased thickness of eggshells of hens fed high Ca.

than in the birds fed only Se, or in the low Ca unsupplemented treatment. The oviducts of the quail fed only Se had lowered CA activities than the controls or MHg fed hens. The Se + MHg treated birds showed similar CA activity in their oviducts as compared to the unsupplemented hens.

DISCUSSION

Because of the high Ca requirement for shell formation (WOLFORD and TANAKA 1970) it was expected that lowered production with thinner shelled eggs would be produced in quail fed the low Ca diet. However, when MHg was present in both the low and high Ca diets, very low egg production with extremely thin shelled eggs occurred. It was obvious that when egg production dropped only the more durable hens were producing thin shelled eggs. This dietary level of MHg cannot be fed without Se since no egg production and high mortality will occur in quail (STOEWSAND et al. 1977).

The high Se residues in liver, kidney and brain of birds fed Se + MHg, as compared with the birds fed Se alone, probably reflects the binding activity of Se with the Hg present in these tissues (GANTHER et al. 1975). Dietary Ca did not seem to have a significant effect on this relationship.

CA catalyzes the hydration of metabolic CO_2 to bicarbonate required to form Ca carbonate of the shell. Inhibition of this enzyme occurs with a linear dose response from administered sulfanilamide causing production of thin shelled eggs in chickens (BENESCH et al. 1944). A reduction in CA apparently was not responsible for the thin shelled eggs produced by the MHg fed quail in this study, indeed their blood CA was even unexpectedly elevated (Table 1). However, since a number of substances can affect Ca deposition and oviposition, both required for adequate shell development (MUELLER and LEACH 1974), MHg may be affecting one or more of these processes. Initial oviposition occurring at a younger age has been shown to happen in Japanese quail fed mercuric chloride (HILL and SHAFFNER 1976).

CA activity in both blood and oviduct of the Japanese quail hens does not seem to be a major factor in eggshell thinning. No significant correlation between chicken eggshell strength and CA activity of the shell gland was reported (HEALD et al. 1968).

It has been observed that DDT causes significant shell thinning in Japanese quail, but only when the diet is deficient in Ca (BITMAN et al. 1969). Our present data show that a lowered Ca diet depressed egg

TABLE 1.

Selenium (Se) Residues in Liver, Kidney and Brain, and Carbonic Anhydrase (CA) activity of Blood and Oviduct of Japanese Quail Hens Fed 2 Calcium (Ca) Diets with Se and Methylmercury (MHg) for 14 weeks.

Dietary Treatments			Selenium Residues[1]			Carbonic Anhydrase (CA) activity[2]	
Ca	Se	MHg	Liver ppm	Kidney ppm	Brain[3] ppm	Blood u/ml	Oviduct $u/mg \times 10^{-2}$
Low	0	0	0.4 ± 0.2	1.0 ± 0.1	0.5	1.7 ± 0.3	3.7 ± 0.4
High	0	0	0.4 ± 0.1	1.2 ± 0.1	0.5	5.0 ± 0.9[4]	4.0 ± 0.6
Low	+	0	7.9 ± 3.0	11.1 ± 1.1	1.9	2.3 ± 0.2	1.8 ± 0.4[5]
High	+	0	7.9 ± 1.9	11.0 ± 0.1	1.3	1.8 ± 0.8	1.6 ± 0.2[5]
Low	+	+	60.5 ± 16.8[4]	28.2 ± 6.0	19.5	4.5 ± 1.2[4]	3.3 ± 0.6
High	+	+	47.6 ± 14.7[4]	22.6 ± 3.3	17.3	4.1 ± 0.8[4]	2.9 ± 0.6

1. Dried tissues, mean ± S.E. of 4 random birds per group.
2. Mean ± S.E. of 6-8 birds per group in CA units.
3. Mean of duplicate analysis on pooled brain tissues within each group.
4. Significantly higher values ($P<0.05$) as compared to the same tissue in other dietary treatments.
5. Significantly lower CA activity ($P<0.05$) than all other oviducts.

production with more broken and shell-less eggs when dietary MHg was present, but egg weight and shell thickness were reduced under either a low or high dietary Ca + MHg regimen. Although Se inhibits acute MHg poisoning (FROST and LISH 1975), it does not appear to counteract MHg induced eggshell thinning in Japanese quail. This occurrence might be considered a rather sub-acute, subtle MHg effect. Susceptibility to MHg causing thin shelled eggs, of course, may not occur in other avian species (PEAKALL and LINCER 1972).

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SUMMARY

A high Ca, semi-purified diet promoted greater egg production with thicker shells in Japanese quail hens. 7 ppm of dietary Se depressed egg weights with no effect on eggshell thickness. The addition of 20 ppm of MHg to either a low or high Ca diet containing Se depressed both egg production and eggshell thickness. Se residues of liver, kidney, and brain in Se-MHg fed quail were significantly higher than in Se treated quail, probably reflecting the ability of Se to bind with Hg. CA, an enzyme required for the formation of the carbonate radical of Ca carbonate in eggshells, was not reduced in the Se-MHg treated Japanese quail hens' blood or oviducts.

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