Embryonic Viability as Influenced by Excess Molybdenum in Chicken Breeder Diets. (29784)

PAUL D. LEPORE* AND R. F. MILLER† (Introduced by R. W. Engel)
Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg

The toxic role of molybdenum in reference to animals of economic importance has been the subject of a recent review(1). In this respect, experiments have been conducted in our laboratory which assessed the toxicity of molybdenum, injected *via* the yolk sac, to the developing chick embryo. The injection technique employed in these studies yielded much interesting information(2,3), but had the following inherent disadvantages: 1. The injected chemicals are deposited in a mass in the yolk material. 2. The injected chemicals are not deposited in the same position relative to the embryo when a series of injections is made. 3. Variations exist in embryonic age.

The purpose of the current study was to investigate the feasibility of using the breeder hen as a means of introducing molybdenum to the developing chick embryo. If this were possible, the hen would distribute a toxic quantity of molybdenum evenly within the egg and thus, the mechanical and age disadvantages ascribed to the injection technique could be overcome.

Preliminary evidence that such a system could be devised has been reported(4). It was observed that feeding laying hens a diet containing 500 ppm of molybdenum would result in the production of eggs containing approximately 12 ppm of molybdenum.

Experimental. Experiment I was designed to determine a dietary molybdenum level which, when fed to laying hens, would result in eggs containing a maximum amount of molybdenum without severely limiting egg production. At 7 months of age, 24 White Rock hens were randomly selected from a larger flock and placed in groups of 6 in floor pens divided by wire partitions. Each of the groups of hens was fed a commercial laying diet containing either 0, 500, 1000 or 2000 ppm of molybdenum added as $Na_2MoO_4 \cdot 2H_2O$ for a 21-day period. During this time, egg production and weekly body weights were recorded. Every egg laid by each hen was broken and the egg contents were analyzed for molybdenum by a thiocyanate method(5).

Determining the influence of the dietary molybdenum level established in Exp. I upon embryonic viability was the objective of Exp. II. Forty 9-month-old hens were used, with 2 groups of 10 receiving the unmodified basal diet and an additional 2 groups of 10 receiving the basal diet plus 500 ppm of molybdenum. The hens were artificially inseminated 6 times with semen pooled from 7 males of the same strain at semiweekly intervals throughout the 21-day experimental period. All eggs were collected and incubated daily. The viability of the embryos was assessed by periodic candling to 19 days of incubation. Each egg and/or embryo was analyzed for molybdenum content either after the occurrence of mortality or after 19 days of incubation.

Exp. III was designed to study the mode of inclusion of molybdenum into the egg by the hen. A separate group of 14 hens was fed the 500 ppm molybdenum diet for a period of 2 weeks. At this time, 5 of the hens were sacrificed and blood, liver, ovary, ova, and oviduct samples were obtained for molybdenum analysis.

Results and discussion. The weight changes and egg production of the hens observed in Exp. I are listed in Table I. It appeared that the hens in each group fed molybdenum lost weight, but analysis of variance coupled with Duncan's multiple range test(6) indicated that this weight loss was significant ($P<.05$) only at the 2000 ppm level of supplementation. It was also noted that a decrease in egg production occurred concomitant with increasing levels of dietary molyb-

* Present address: U. S. Dept. of Agriculture, Animal Husbandry Research Division, ARS, Beltsville, Md.
† Deceased, February 1963.

TABLE I. Weight Changes and Egg Production of Hens Fed Molybdenum for a 21-Day Period.

Treatment	Wt* change (g)	No. of* eggs per hen
Basal ration	+ 62[a]	15.6
" " + 500 ppm Mo	− 141[a]	13.3
" " + 1000 " "	− 148[a]	8.0
" " + 2000 " "	− 449[b]	2.8

* Means of 6 hens per dietary treatment. Those means with the same superscript are not significantly different ($P < .05$).

denum. Although these data were not analyzed statistically, it is evident that only a slight decrease in egg production occurs when 500 ppm of molybdenum are fed. At this level, the molybdenum fed hens laid 15% fewer eggs than the controls. The decreases noted at 1000 and 2000 ppm of molybdenum were 50% and 80%, respectively.

The relationship between egg molybdenum concentration and length of time of feeding the experimental diets can be seen in Fig. 1. Regression analysis permitted the fitting of the polynomial functions depicted. Analysis of variance of the data indicated a highly significant influence of dietary molybdenum level upon the molybdenum concentration of the eggs. In addition, the curves obtained for each level of supplementation had significant linear and quadratic components. The peculiar shape of the curve representing the response at 2000 ppm of molybdenum was caused by the cessation of egg production by these hens. The curve obtained at the 500 ppm level was of major interest in that it depicted a plateauing of the egg molybdenum concentration at 16 ppm after approximately 2 weeks of the dietary treatment. It will be recalled from Table I that this level had the least effect upon egg production. Possibly, the slight discrepancy between our data and that previously reported (4) may be explained by breed and seasonal differences.

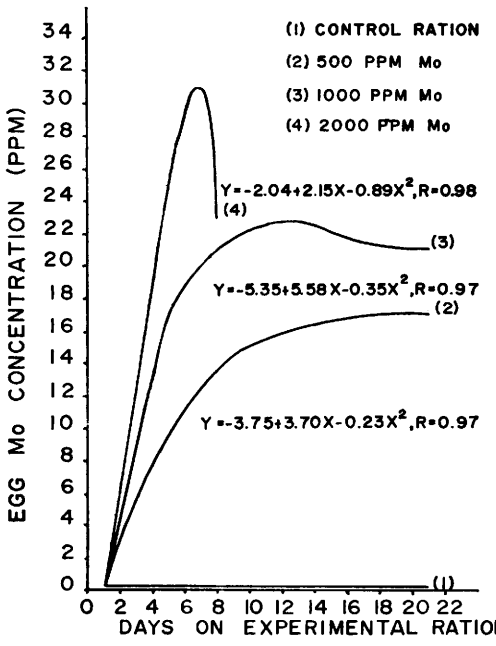

FIG. 1. Egg molybdenum concentration as influenced by dietary molybdenum level and length of feeding period.

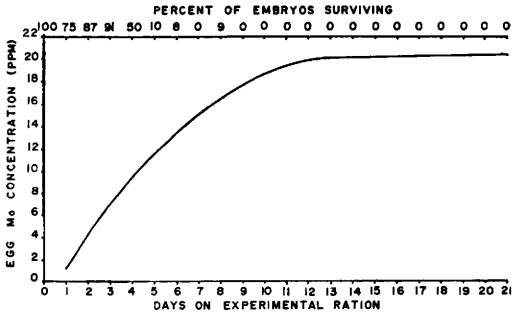

FIG. 2. Embryonic viability as influenced by egg molybdenum concentration and length of feeding 500 ppm of molybdenum to breeder hens.

The results obtained in Exp. I prompted the use of the 500 ppm dietary level of molybdenum for assessment of embryonic molybdenum toxicity.

In Exp. II, replicate groups of 10 hens were fed the basal diet with and without 500 ppm of molybdenum. Egg production through the 21-day period was similar to that observed in Exp. I. The molybdenum fed hens laid about 80% as many eggs as the control hens.

The data from the replicate hens were pooled in order to obtain the information depicted in Fig. 2. As in the previous experiment, a curve can be fitted to represent the relationship between egg molybdenum concentration and duration of feeding 500 ppm of molybdenum. In contrast to the 16 ppm plateau level observed in Exp. I, the egg

TABLE II. Molybdenum Concentration of Various Tissues of Hens Fed 500 ppm of Molybdenum.

Tissue	Mo concentration*
Blood	29 ± 9
Liver	42 ± 11
Ovary	150 ± 13
Oviduct A†	51 ± 18
Oviduct B‡	148 ± 42
Maturing ova	
1 largest	72 ± 13
2 "	76 ± 14
3 "	72 ± 16
4 "	73 ± 23
5 "	91 ± 45
6 "	86 ± 29
7 smallest	96 ± 16
Fresh egg yolk	85 ± 11
Fresh egg albumen	31 ± 12

* Mean ± s.d. of 5 observations. Blood concentration is reported as μg/ml. For the other tissues, it is reported as ppm on a dry weight basis.
† Infundibulum, magnum and isthmus.
‡ Uterus and vagina.

molybdenum concentration in this experiment plateaued at 20 ppm. If age of the hen and season are important influences on molybdenum deposition, the between experiment differences can easily be resolved. Exp. II was conducted in mid-winter with hens 2 months older than those in Exp. I.

The percentage of surviving embryos is listed across the top of Fig. 2. By comparing these percentages with the egg molybdenum concentration observed on the same day, it appears that all of the embryos die when egg molybdenum concentration is in excess of 17 ppm. This embryonic mortality, caused by molybdenum deposited in the egg by the hen, did not occur at a specific developmental age. Ten percent of the embryos died during the first 5 days of incubation, 15% on the sixth day, 35% on the seventh day and 40% during the eighth through the twelfth days. No special significance could be attached to this sporadic lethal response.

This experiment indicated that the feeding of sub-toxic quantities of molybdenum to breeder hens would result in the production of eggs containing lethal quantities of this element. Whether a similar system could be devised to study the embryonic toxicity of other minerals is a subject for further study.

The molybdenum concentrations of the tissues from the molybdenum fed hens in Exp. III are listed in Table II. The oviduct was analyzed in 2 portions, one composed of the infundibulum, the magnum and the isthmus and the other composed of the uterus and the vagina. The ovary plus immature ova and the lower part of the oviduct had higher molybdenum concentrations than any of the other tissues tested. By comparing molybdenum concentrations of the series of maturing ova with the laid egg yolk, it appeared that about 85% of the molybdenum in the yolk of freshly laid eggs was deposited in the ovary. The sequence of events leading to the inclusion of molybdenum into the egg by the hen may be summarized as follows: The major portion of the molybdenum in the egg is incorporated during maturation of the ova; the yolk increases slightly in molybdenum concentration as it traverses the oviduct; the albumen does not attain as high a molybdenum concentration as the yolk due to the lowered concentration of molybdenum in the magnum as opposed to the ovary; the uterus had a high molybdenum concentration, suggesting that the egg shell be considered in future work of this nature.

Summary. Embryonic molybdenum toxicity may be studied through use of the breeder hen. The feeding of 500 ppm of molybdenum to hens for longer than 2 weeks results in eggs having a concentration of between 16-20 ppm of molybdenum. This dietary level does not limit egg production too severely (ca. 20% reduction) but does result in the death of all embryos during the incubation period. Analysis of the tissues from the molybdenum fed hens showed that the ovary and the uterus had the highest concentrations of molybdenum (150 and 148 ppm, respectively).

1. Miller, R. F., Engel, R. W., Fed. Proc., 1960, v19, 666.
2. Lepore, P. D., Miller, R. F., ibid., 1961, v20, 296.
3. Lepore, P. D., Miller, R. F., Siegel, H. S., Poultry Sci., 1962, v41, 1047.
4. Motzok, I., Arthur, D., Branion, H. D., ibid., 1957, v36, 1144.
5. Evans, H. J., Purvis, E. R., Bear, F. E., Anal. Chem., 1950, v22, 1568.
6. Duncan, D. B., Biometrics, 1956, v1, 11.

Received October 1, 1964. P.S.E.B.M., 1965, v118.