MOLYBDENUM TOXICITY IN THE DEVELOPING CHICK EMBRYO

Dissertation submitted to the graduate faculty
of Virginia Polytechnic Institute
in candidacy for the degree of

DOCTOR OF PHILOSOPHY

in

Biochemistry and Nutrition

by

PAUL DOMINICK LEPORE

October, 1962

Blacksburg, Virginia

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction and Literature Review

The identification of molybdenum as an integral part of the prosthetic group of the enzyme xanthine oxidase in 1953, stimulated intensive research concerning the biological significance of this mineral (9). The research conducted to data may be divided into two broad categories, that concerned with the role of molybdenum as an essential mineral in animal nutrition; and that concerned with the toxicity observed in animals consuming diets containing excessive quantities of molybdenum.

Several lines of evidence serve to support the essential status of molybdenum in animal nutrition. It has been demonstrated that the liver enzymes, xanthine oxidase (9) and aldehyde oxidase (18) are molybdoflavoproteins. Further, the feeding of diets low in molybdenum or protein causes a depletion of both molybdenum and xanthine oxidase from the liver and intestine of experimental animals (9,12). Although these facts indicate the essential role of molybdenum, it has not been conclusively demonstrated that either of these enzymes are necessary for the well-being or normal physiology of the animal.

The use of tungsten, a molybdenum antagonist, has provided further evidence for the essentiality of molybdenum. De Renzo observed that tungstate competitively inhibited molybdate in the xanthine oxidase system *in vitro* (8).

Higgins *et al*. extended this observation *in vivo* utilizing both rats and chicks as experimental animals (12). These workers observed no adverse effects from the feeding of tungsten in the W/Mo ratio of 2000:1 in the rat, despite the fact that both liver and intestinal xanthine oxidase activity was markedly depressed. In contrast, the feeding of tungsten in this ratio to chicks resulted not only in depressed tissue levels of molybdenum and xanthine oxidase, but also in a slight depression in growth as well as a 25% incidence of mortality. The cause of this species-specific response was attributed to the increased importance of the xanthine oxidase system in the chick. An interesting approach to the tungsten-molybdenum problem was provided by Teekel and Watts. These workers observed that chicks from breeder hens receiving tungsten (500 ppm) in their ration grew at significantly slower rates than did the control chicks from non-supplemented hens (30). That this growth depression was caused by a carry-over of tungsten from dam to chick was demonstrated when the inclusion of 100 ppm of molybdenum into the diets of the chicks from the tungsten supplemented hens resulted in a growth response equivalent to the chicks from the non-supplemented hens (31). Although these studies appear conclusive, the question remains whether tungsten produces a true molybdenum deficiency or whether it produces a toxicity which can be conveniently reversed by molybdenum supplementation.

The final line of evidence, and what would seem to constitute direct proof of the essentiality of molybdenum, is derived from the work of Couch et al. (14,25,26,33). These workers obtained a 13-18% increase in growth in chicks and poults fed a partially purified diet to which molybdenum had been added. However, certain objections can be raised concerning these experiments. The growth response was obtained by increasing the concentration of molybdenum in the basal ration from 1.00 to 1.03 ppm. It is difficult to comprehend how such a small change in the basal ration could result in such a profound difference in rate of growth. Second, no stoichiometric relation existed between the magnitude of the growth response and the dietary concentration of molybdenum. Third, most of the experiments were conducted with single replicate groups. Finally, and most important, the results of these experiments have yet to be confirmed by workers in other laboratories. In conclusion, it is generally accepted that molybdenum is a dietary essential, but direct evidence is lacking.

The literature concerning molybdenum toxicity has been recently reviewed (21).

In poultry, the physical symptoms of molybdenosis are manifested by a decreased rate of growth and anemia. Thus, Kratzer reported a 25% growth depression in growing chicks and poults obtained by the inclusion of 300 ppm of molybdenum into a practical type basal ration (13). Two hundred ppm

(1) and 500 ppm (4,20,32) of molybdenum have also been shown to result in a slight growth depression in growing chicks. nemia, however, does not occur at these low levels of molybdenum supplementation. It has been reported that levels of molybdenum in excess of 2000 ppm are necessary to produce an observable anemia (1,5,7).

The efficacy of copper in alleviating molybdenosis has been studied. Kratzer could partially alleviate the growth depression caused by 300 ppm of molybdenum by supplementing the diets with 100, 200, or 300 ppm of copper (13). This observation was later confirmed in that the growth depression caused by 200 and 350 ppm of molybdenum could be overcome by supplementation with 100-500 ppm of copper (1). It was further observed that no level of copper used (up to 500 ppm) could overcome the growth depression caused by diets containing in excess of 500 ppm of molybdenum. In an unusual experiment designed to study the copper-molybdenum interrelationship, Miller and Denton could prevent the depigmentation of feathers produced by a diet containing both molybdenum and thiosulfate by the addition of copper to the ration (19). However, neither Davies _et al_. (4) nor Miller and Denton (20) could overcome the toxic effects of excess dietary molybdenum at any level of copper supplementation attempted (level-200 ppm).

That inorganic sulfate is effective in alleviating molybdenosis has been demonstrated. Davies _et al_. reported that sulfate could reduce the growth depression caused by 2000 ppm of molybdenum (5,6). Miller and Denton overcame half the growth depression caused by 2,250 ppm of molybdenum by the addition of 13,200 ppm of sulfate to the diet (20). Davies et al. have subsequently reported that sulfate added to chick diets at twice the concentration of molybdenum alleviated all the physical symptoms of the toxicity (growth depression and anemia) (7).

In studying the specificity of the sulfate effect, Miller and Denton observed that methionine was also partially effective as a mitigating agent of molybdenosis in chicks (20). Other sulfur containing compounds such as taurine, sodium bisulfite, sodium sulfite, and sodium sulfide were only partially as effective as methionine. A later report by other workers, however, failed to confirm the methionine effect on molybdenum toxicity (7).

The effect of excess molybdenum has also been studied in the developing chick embryo. Thus, it has been reported that the $L.D._{50}$ of molybdenum injected into the yolk sac of chick embryos of four days of incubation was 0.80 mg (27). At eight days of incubation, the $L.D._{50}$ was 0.70 mg. These workers observed no gross pathological effects in embryos of 19 days of incubation which had been injected with sub-lethal amounts of molybdenum.

The results of experiments conducted at this laboratory involving the assessment of the toxicity of molybdenum injected into the yolk sac of developing chick embryos have been reported (15,16,17). It was observed that the lethality of the injected molybdenum to the chick embryo decreased as embryonic age advanced. Further, the $L.D._{100}$ of molybdenum for White Rock embryos of four days of incubation was determined to be 0.50 mg of molybdenum. The lethal response elicited by this amount of molybdenum was noted in the death of all injected embryos by the third day post injection. Also, if various salts (copper chloride, sodium sulfate, sodium chloride, potassium chloride and potassium sulfate) were injected concurrent with the lethal molybdenum dose, it was observed that about 40% of the embryos survived. It was also reported that chicks hatched from embryos injected with sublethal amounts of molybdenum exhibited poorer growth when fed a basal diet than did the chicks from the corresponding saline injected control embryos. It was postulated that the carryover of molybdenum from embryo to chick was sufficient to depress the growth of the chicks.

Despite the interesting results obtained utilizing the injection technique, it became apparent that this technique suffers from several inherent disadvantages. One is always confronted with a basal level of mortality caused by occasional faulty injection technique. Also, the varying geometry of the injection may serve to confound results. As such, an injection made more distal to the embryo than another at the same dose level could result in a different response. Finally, and most important, the injected chemical is deposited in a mass in the yolk material. Thus, local physical and chemical reactions occurring, as it were, in the embryonic nutritional supply, could greatly affect toxicity but would be totally unrelated to the physiology of the developing embryo.

These considerations prompted investigations using the laying hen as a means of introducing molybdenum into the egg. This was shown to be possible by the initial study of Motzok _et al_. (23). These workers obtained eggs containing 1.0, 2.2, 5.4, and 12.2 ppm of molybdenum from hens fed 0, 50, 100, and 500 ppm of molybdenum, respectively.

Therefore, the objectives of the following research were: to determine the feasibility of utilizing the laying hen as a source of molybdenum containing eggs; to determine if these eggs were suitable for studying molybdenum toxicity in the developing chick embryo; to determine the distribution of molybdenum in these eggs; to compare the lethality of injected molybdenum versus molybdenum introduced via the laying hen; and, finally, to reinvestigate the growth depression observed in chicks which had been injected with molybdenum during their embryonic development.

Experiment I - The Dose Response of Hens to Dietary Molybdenum as Reflected by the Egg Molybdenum Concentration

In order to evaluate the laying hen as a source of molybdenum containing eggs, it was first necessary to establish the relation between the dietary and the egg molybdenum concentrations. It had been estimated previously that the $L.D._{100}$ of molybdenum for White Rock embryos of one day of incubation ranged from 10-20 ppm on a fresh egg weight basis (15). This observation, coupled with the assumption that injected molybdenum and molybdenum included into the egg by the laying hen are equally as toxic to the developing chick embryo, provided the basis for Experiment I.

Thus, it was desired to determine a dietary molybdenum level which, when fed to laying hens, would be relatively non-toxic to the hen, but would result in the production of eggs having a molybdenum concentration within 10-20 ppm.

Experimental

The hens used in this study were the fourth generation of adult White Rocks selected for wide breast angle which were developed by Siegel (28). At 215 days of age, 24 of these hens were randomly selected from a larger flock and placed in groups of six in floor pens divided by welded wire partitions. The pens were littered with wood shavings and equipped with trap nests and automatic watering devices. Galvanized feeders and oyster shell dispensers were attached to the wire divider of each pen.

Prior to the initiation of the experimental period, the hens were acclimatized for two weeks while consuming the unmodified basal ration. The practical basal ration used was provided and mixed to conform to V.P.I. specifications by the Southern States Cooperative. The composition of the ration is listed in Table 1. For all hen experiments, feed, water, and oyster shells were provided *ad libitum*. Molybdenum was added to the diets as an aquaeous solution of sodium molybdate dihydrate. The four diets consisted of the basal ration to which had been added 0, 500, 1000, and 2000 ppm of molybdenum, respectively. The experimental procedure involved the feeding of these diets to the hens for a 21-day period, during which time egg production and weekly weight gain were recorded. The eggs from each hen of each day of this period were collected and analyzed for molybdenum content by the thiocyanate method of Evans *et al*.(11). After the experimental period, the hens receiving 500 ppm of molybdenum in their ration were again fed the basal ration in order to afford an estimate of the recovery rate of these hens from the effects of the dietary molybdenum.

Results and Discussion

The weight gain data are summarized in Table 2. Analysis of variance of these data indicated a highly significant effect of diet on the total weight change of the individual hens. Separation of the mean weight changes by using Duncan's

Table 1.

Laying Hen Basal Ration

Ingredient	lb./ton
Yellow corn meal	1220.5
Pulverized oats	200.0
Wheat flour middlings	100.0
Wheat bran	100.0
Alfalfa meal (17% crude protein)	50.0
Fish meal (60% crude protein)	40.0
Meat and bone scraps (50% crude protein)	100.0
Soybean oil meal (50% crude protein)	160.0
Salt, iodized	8.0
Ground limestone (98% $CaCO_3$)	10.0
Deflourinated phosphate (31% Ca, 18% P)	6.0
Manganese sulfate (24% Mn)	0.5
Vitamin A supplement (10,000 I.U. per gram)	1.0
Vitamin D_3 supplement (6,000 I.C.U. per gram)	0.5
Multivitamin mixture*	2.0
Vitamin B_{12} supplement (6 mg B_{12} per lb.)	1.0
Choline chloride	0.5

* One pound contains: 2.0 g riboflavin, 4.0 g calcium pantothenate, 10.0 g niacin, 0.25 g menadione sodium bisulfite, 0.003 g Vitamin B_{12}.

Table 2.

Weight Changes in Laying Hens Accompanying the Feeding of Graded Levels of Dietary Molybdenum

Treatment	The number of days on treatment 0	7	14	21	Mean Weight Change[2]
Control	2648 ±61[1]	2668 ±79	2753 ±76	2710 ±83	+62[a]
500 ppm Mo	2746 ±47	2782 ±49	2729 ±45	2605 ±49	-141[a]
1000 ppm Mo	2975 ±49	2953 ±59	2893 ±56	2827 ±60	-148[a]
2000 ppm Mo	2943 ±57	2688 ±62	2596 ±53	2494 ±52	-449[b]

1 Average weight in grams of six hens, ± s.d.
2 Means with the same superscript are not significantly different. ($P \leq 0.01$)

multiple range test (10) at the 1% level of probability indicated that under the conditions of the experiment, more than 1000 ppm of dietary molybdenum was necessary to produce a significant weight loss in the laying hen.

The influence of these diets on egg production is demonstrated in the summarized data in Table 3. Although the data have not been statistically analyzed, certain trends are evident. At both 1000 and 2000 ppm of molybdenum, a profound decrease in egg production was noted. This is especially manifest at 2000 ppm in that all hens on this treatment were out of production after two weeks of feeding the experimental diet. However, comparison of the hens receiving 500 ppm of molybdenum with the control hens indicated only a 15% decrease in egg production in this group.

Regression analysis by the method of least squares of the average egg molybdenum concentration versus the number of days on experiment permitted the fitting of the polynomial functions depicted in Figure 1. The eggs from the hens fed the control diet demonstrated no fluctuation in molybdenum concentration with respect to time. However, analysis of variance of the data obtained from the eggs of the hens fed the experimental diets indicated a highly significant effect of length of time of feeding of the molybdenum-containing diets on the molybdenum concentration of the eggs. Further, all three curves had significant linear and quadratic components. The following summarizes the properties of the curves:

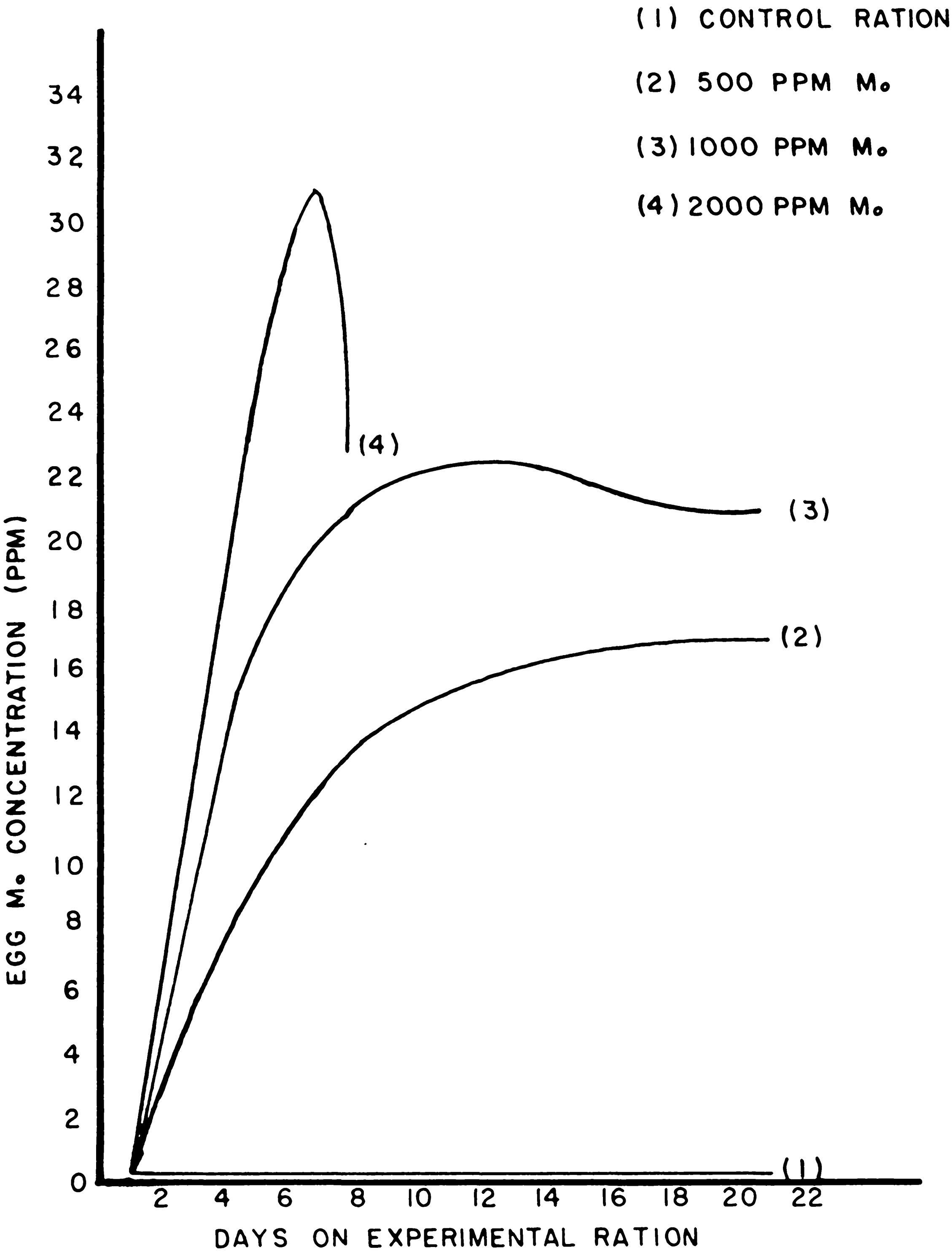
Table 3.

Egg Production of Laying Hens Fed Diets
Containing Graded Levels of Molybdenum

Treatment	Collection Period 1st week	2nd week	3rd week	% of Controls
Control	5.33*	5.00	5.33	100.0
500 ppm Mo	4.17	4.67	4.50	85.2
1000 ppm Mo	2.83	3.00	2.17	51.1
2000 ppm Mo	2.17	0.60	0.00	17.7

* Average number of eggs per hen per week.

FIGURE 1. THE EGG MOLYBDENUM CONCENTRATION AS INFLUENCED BY DIETARY MOLYBDENUM LEVEL AND LENGTH OF FEEDING PERIOD.



Treatment	Equation	R (multiple correlation coefficient)
500 ppm Mo	$Y = -3.7517 + 3.7048X - 0.2276X^2$	0.9659
1000 ppm Mo	$Y = -5.3488 + 5.5769X - 0.3549X^2$	0.9713
2000 ppm Mo	$Y = -2.0428 + 2.1451X - 0.8937X^2$	0.9804

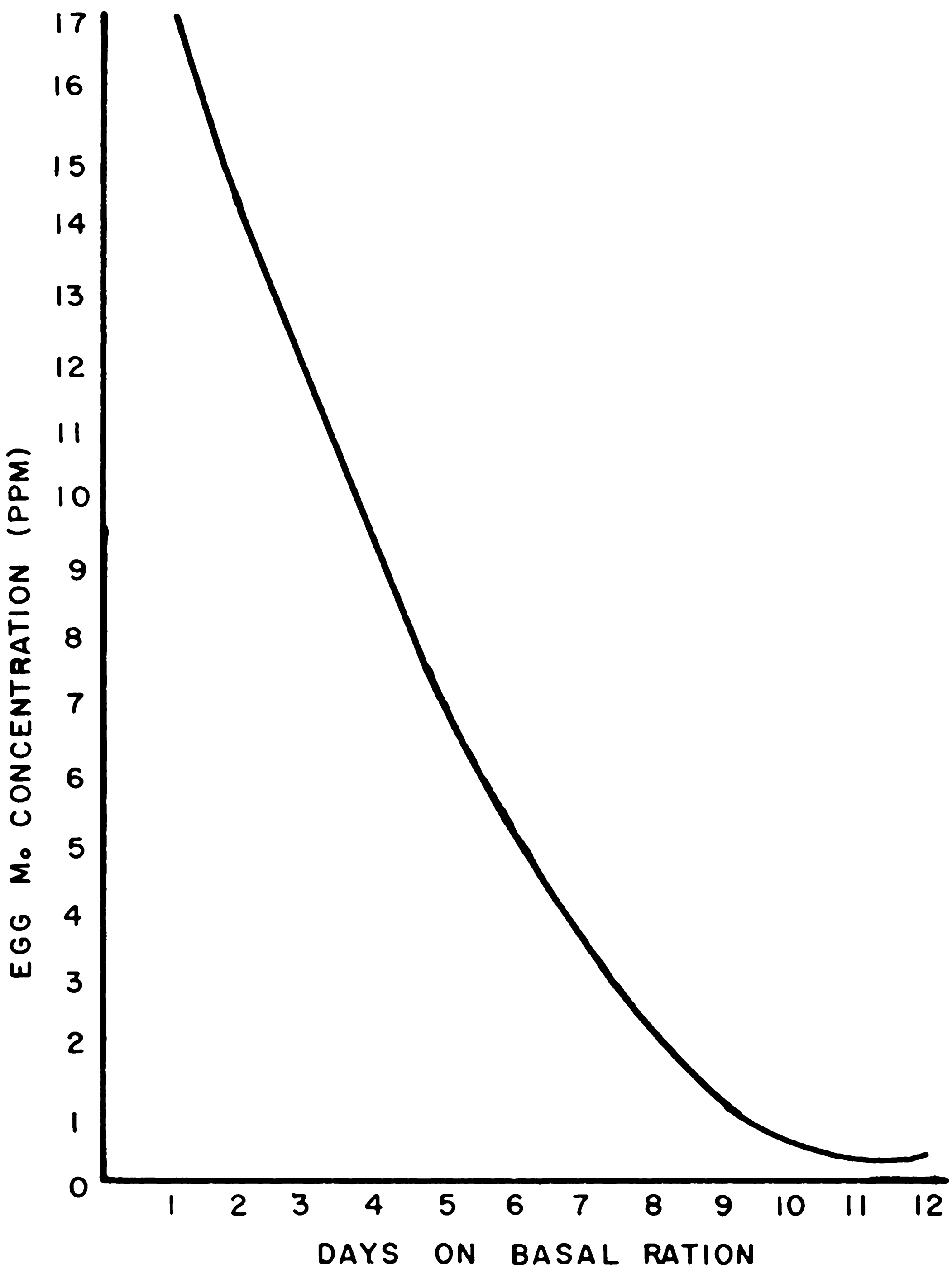
The curve obtained at the 500 ppm dietary level was of major interest in that it depicted a plateauing of the molybdenum concentration in the egg at about 16.4 ppm after 14 days of the dietary treatment.

The peculiar shape of the curve depicting the response at the 2000 ppm dietary level was due in part to the cessation of egg production by these hens after nine days of feeding and also to the paucity of observations obtained on the eighth day of feeding.

A similar analysis of the data obtained on the eggs from the hens fed 500 ppm of molybdenum after cessation of the dietary treatment yielded the curve depicted in Figure 2. It is pertinent to observe that after only nine days of consuming the basal ration, these hens were producing eggs containing essentially control quantities of molybdenum. Thus, it appeared that the tissues of the hens fed the basal ration after consuming a molybdenum-containing diet were rapidly depleted of their molybdenum content.

From the above observations, it was concluded that the feeding of a diet containing 500 ppm of molybdenum to the laying hen would result in the production of eggs that could be used in the study of molybdenum toxicity in the developing chick embryo.

FIGURE 2. THE DECLINE IN EGG MOLYBDENUM CONCENTRATION OCCURING WITH REPLACEMENT OF MOLYBDENUM FED HENS ON THE BASAL RATION.



Summary

A practical ration containing 0, 500, 1000, and 2000 ppm of added molybdenum was fed to White Rock laying hens. The 1000 and 2000 ppm levels of supplementation resulted in a decrease in egg production and/or weight loss in the hens.

Supplementation at 500 ppm of molybdenum, however, resulted in only a slight decrease in egg production with no appreciable weight loss. Further, the egg concentration of molybdenum plateaued at 16.4 ppm after 14 days of feeding the experimental ration.

A recovery experiment indicated that the molybdenum concentration of the egg approached control values nine days after cessation of feeding the molybdenum supplemented ration.

Experiment II - The Toxicity of Molybdenum Included in the Egg by the Laying Hen to the Developing Chick Embryo

The previous study indicated that of the three dietary treatments attempted, the 500 ppm molybdenum level proved to be the most amenable for the study of molybdenum toxicity in the developing chick embryo.

This study was extended by Experiment II which was designed to assess the toxicity of molybdenum included in the egg by the laying hen to the developing chick embryo. Further, it was desired to measure several biochemical parameters on the embryonating eggs from these hens.

Experimental

The basic design of the experiment involved the feeding of the basal ration described for Experiment I both with and without 500 ppm of molybdenum to two replicate groups of ten White Rock hens. These hens were artifically insemenated throughout the 21-day experimental period. The eggs from these hens were incubated to determine viability and analyzed to determine molybdenum content.

The husbandry practices were similar to those described for Experiment I. Forty hens of 280 days of age were placed in groups of ten into four floor pens. As previously, a two week acclimatization period was permitted. Seven males of the same age and stock, maintained in raised individual wire-floored cages and fed the stock basal ration, were used to provide semen for the artificial insemenation. The hens were insemenated twice in the week prior to the initiation of the experiment, and once weekly, thereafter.

On each day of the experimental period, each egg from each hen was collected and placed into a Humidaire incubator maintained at the requisite temperature and humidity for the development of the embryos. The viability of the embryos was assessed by periodic candling up to 19 days of incubation. After the viability had been determined, each egg was analyzed for molybdenum content as previously described.

After the completion of the 21-day experimental period, the hens were fed the experimental diets in order to provide a source of eggs and embryos for further experiments. Succinoxidase was determined on embryo homogenates by the method of Potter (24). The residual eggs were separated into yolk and albumen fractions, frozen, and stored at -20° until use in the chemical fractionation studies.

Results and Discussion

The egg production data observed in this experiment are summarized in Table 4. It should be noted that the egg production of both the control and the treated hens was below that observed in Experiment I. This was most probably due to the colder weather conditions prevailing when Experiment II was conducted. However, again only a slight

Table 4.

Egg Production of Hens Fed a Basal Diet with and without 500 ppm of Molybdenum

Treatment	Collection Period 1st week	2nd week	3rd week	% of Controls (reps pooled)
Control (rep 1)	3.70*	3.80	3.80	100
Control (rep 2)	4.20	3.90	3.90	
500 ppm Mo (rep 1)	3.90	3.60	3.10	79
500 ppm Mo (rep 2)	3.60	2.20	2.10	

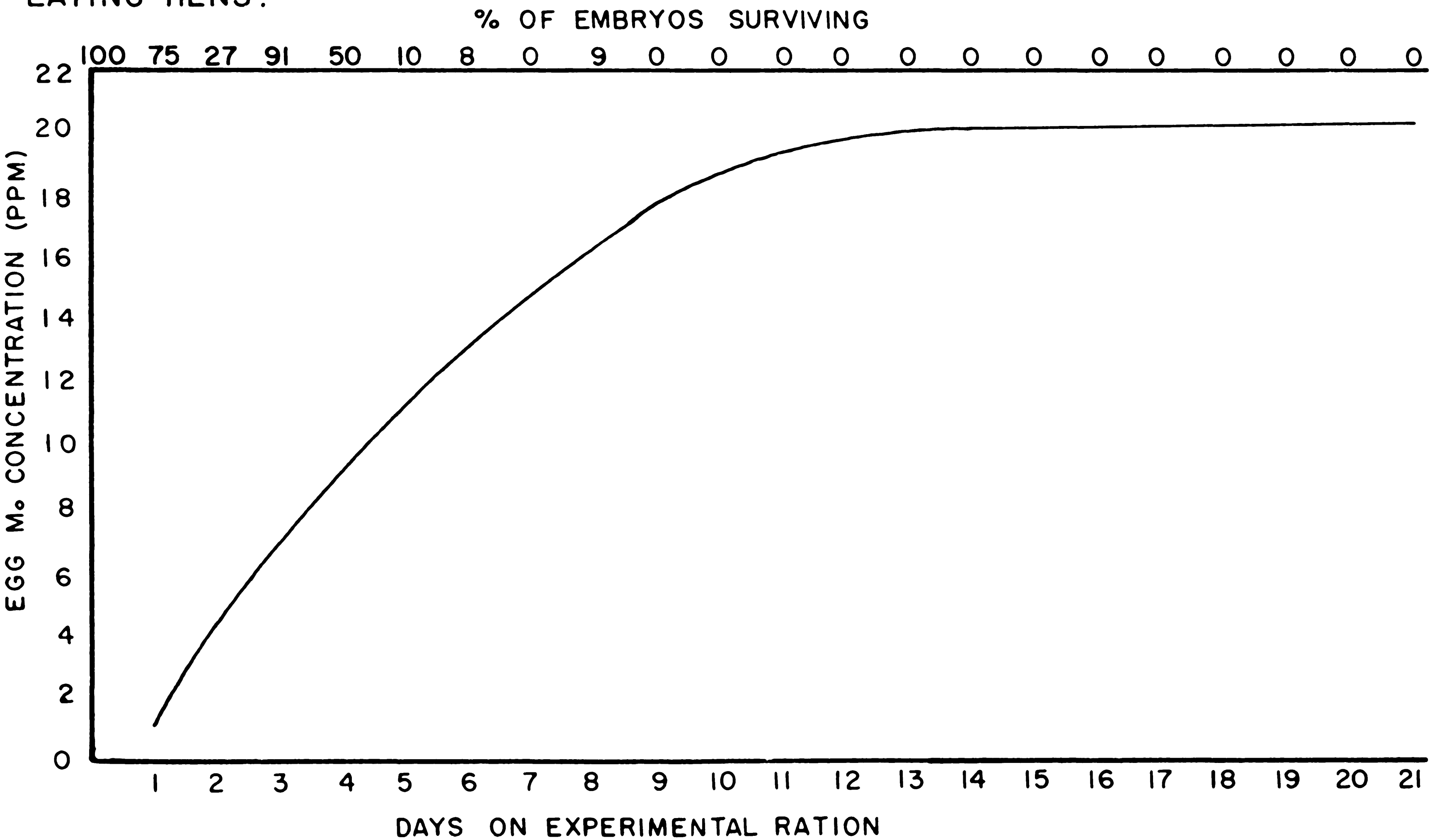
* Represents the average number of eggs per hen per week from a group of ten White Rock hens.

depression (ca. 20%) in the average egg production of the molybdenum fed hens compared with the control hens was noted.

The curve in Figure 3, fitted by the method of least squares, illustrates the relationship between the egg molybdenum concentration and the length of feeding of the molybdenum containing diet. For simplicity, the analyses of the eggs from each replicate group of hens were pooled. The polynomial equation of the curve had significant linear and quadratic components. The equation of the curve is: $Y = -2.2547 + 3.6878X - 0.1967X^2$, and the R obtained is 0.9796. The percent of embryonic survivals on a particular treatment day is listed across the top of the graph. Thus, considering both the embryo survival and the molybdenum concentration data, it can be seen that after eight days of molybdenum feeding, corresponding to an egg molybdenum concentration of about 17 ppm, all embryos died. This egg molybdenum concentration estimated the $L.D._{100}$ of molybdenum included by the laying hen into the egg to the developing chick embryo. It was surprising that this value agreed quite closely to the $L.D._{100}$ of injected molybdenum (15). One would assume that the laying hen would provide for the continuation of the species, even in the presence of an unnatural challenge.

By candling the embryonating eggs, it was observed that the lethal effects of the molybdenum were exerted mostly

FIGURE 3. EMBRYONIC SURVIVAL AS INFLUENCED BY EGG MOLYBDENUM CONCENTRATION AND LENGTH OF FEEDING A BASAL RATION CONTAINING 500 PPM MOLYBDENUM TO LAYING HENS.



during the 7th day of incubation. Thus, 10% of the embryos died from the 1st to the 5th day of incubation, 15% on the 6th day, 35% on the 7th day, and 40% during the 8th to the 12th days. No special significance could be attached to this sporadic lethal response.

To investigate the lethal response further, succinoxidase assays were conducted on both control and molybdenum treated embryos of six days of incubation. Table 5 summarizes the results of this investigation. Analysis of variance of these data within trials indicated that there was no difference in enzyme activity between the control and the molybdenum treated embryos ($P \leq 0.01$).

Summary

Replication of the effect of 500 ppm of dietary molybdenum on egg production in the laying hen again resulted in only a small decrease (20%).

The $L.D._{100}$ of molybdenum included by the laying hen in the egg to the developing chick embryo was estimated to be 17 ppm.

No significance could be attached to the time of death of the embryos.

There was no difference between succinoxidase activity of the control and the molybdenum treated embryos.

Table 5.

Succinoxidase Activity of Whole Homogenates of Control and Molybdenum Treated Six Day Embryos

Trial Number	Control No. Assayed	Control Activity	Mo treated No. Assayed	Mo treated Activity
1	5	3.478* ±.635	5	3.637 ±.285
2	5	3.330 ±.330	5	3.204 ±.494
3	5	3.756 ±.346	6	3.998 ±.567
4	6	2.700 ±.397	4	2.861 ±.335

* Mean succinoxidase activity in μlO_2/hr./mg protein ± s.d. The assays were conducted using the Warburg constant volume respirometer. The contents of the incubation mixture were: 0.5 ml of a 20% whole embryo homogenate, 100 μm of Na_2HPO_4 adjusted to pH 7.4, 166 μm of succinic acid adjusted to pH 7.4, 0.04 μm of cytochrome c, and 1.3 μm of both $CaCl_2$ and $AlCl_3$. The center well contained 0.2 ml of 10% KOH. Respiration was measured for one hour at 37 degrees. Protein assays were conducted by a modified Folin method (29).

Experiment III - The Decrease in the Lethal Response in Embryos from Hens Fed 500 ppm of Dietary Molybdenum Over an Extended Period of Time and the Mode of Inclusion of Molybdenum in the Egg

In the course of the previous studies, it was noted that some of the embryos from the hens consuming the molybdenum containing ration for longer than three months survived. The cause of this survival was investigated in the following experiment.

Further, it was desired to ascertain the mode of inclusion of molybdenum in the egg by the laying hen.

Experimental

It was postulated that the observed decline in the number of embryos dying was mediated by a decrease of molybdenum concentration in the egg caused by erroneous ration mixing or some other more subtle factor. To test this, molybdenum analyses were conducted on both the diets and the eggs of the hens fed molybdenum for an extended period of time.

For the study of the mode of inclusion of molybdenum, a separate group of White Rock hens of 393 days of age were fed the molybdenum containing ration for a period of two weeks. At the end of this time, five of the hens were sacrificed and tissue samples were removed for molybdenum analysis. Blood was analyzed directly, whereas, liver, ovary, oviduct and immature ova were dried for a 48-hour period prior to analysis. The oviduct was divided into two portions for analysis: the albumen secreting region, composed of the infundibulum, the magnum, and the isthmus; and the shell forming region, composed of the uterus. The vagina was included with the uterus for the analyses.

Results and Discussion

Two different mixes of feed analyzed 510 and 569 ppm of molybdenum, respectively. Thus, an error in feed mixing was ruled out as a cause of the decrease in the lethal response in the embryos from the hens fed the molybdenum containing ration for an extended period of time. However, the decrease in lethal response was mediated through a decrease in the molybdenum concentration of the egg as shown in Table 6. If 17 ppm of molybdenum is taken as the $L.D._{100}$, 11 of the 16 eggs analyzed did not have the requisite molybdenum concentration to elicit the lethal response. Although the cause for this reduced molybdenum concentration is unknown, it may be attributable to a combination of factors such as season, age of the hens, or it may reflect a true adaptation mechanism for survival of the species.

Table 7 summarizes the molybdenum analyses concerned with the mode of inclusion of molybdenum in the egg. It is pertinent to note that the ovary and the uterus are the tissues with the highest concentration of molybdenum. Further,

Table 6.

Molybdenum Concentration of Eggs from Hens Fed 500 ppm of Dietary Molybdenum for a Three Month Period

Egg Number	Mo Concentration	Egg Number	Mo Concentration
1	20.70*	9	10.34
2	18.36	10	12.54
3	15.67	11	17.06
4	13.02	12	11.37
5	8.84	13	9.32
6	22.38	14	15.74
7	17.33	15	11.10
8	10.23	16	14.86

* ppm molybdenum on a fresh weight basis.

Table 7.

Molybdenum Concentration of Various Tissues of
Hens Fed 500 ppm of Dietary Molybdenum

Tissue	Mo Concentration
Blood	29 ± 9*
Liver	42 ±11
Ovary	150 ±13
Oviduct-albumen secreting region	51 ±18
Oviduct-shell forming region	148 ±42
Immature ova (in order of decreasing size)	
1	72 ±13
2	76 ±14
3	72 ±16
4	73 ±23
5	91 ±45
6	86 ±29
7	96 ±16
Fresh egg yolk	85 ±11
Fresh egg albumen	31 ±12

* The mean ± s.d. of five observations. The blood concentration of molybdenum is reported as μg/ml of whole blood. For the other tissues it is reported as ppm on a dry weight basis.

by comparing the molybdenum concentration of the immature ova with the laid egg yolk, it can be seen that over 80% of the molybdenum in the laid egg yolk arises from the ovary. The difference in concentration between the ovary and the immature ova suggests that a simple diffusion mechanism could account for the passage of molybdenum from ovary to ova. Since the laid egg albumen had only one third the molybdenum concentration of the laid egg yolk, it was not surprising to observe that the albumen secreting portion of the oviduct had only one third the molybdenum concentration of the ovary. The observation that the uterus had a relatively high molybdenum concentration suggests that in future studies, the molybdenum concentration of the shell should be considered. Summarizing the sequence of events, it appears that: The major portion of the molybdenum in the egg is included during maturation of the ovum in the ovary; The yolk increases in concentration as it passes through the oviduct; The albumen does not attain as high a molybdenum concentration as the yolk, due to the lower concentration of molybdenum in the magnum as opposed to the ovary; The uterus was also high in molybdenum concentration which indicates a study of the molybdenum level of the shell in future work.

Summary

The decrease in the lethal response in embryos from hens fed 500 ppm of dietary molybdenum over an extended period of time was found to be mediated through a decrease in the egg concentration of molybdenum. This decrease in egg concentration was not a result of an error in feed mixing.

The results of the mode of inclusion of molybdenum in the egg indicated that a major portion of the molybdenum in the egg is included during the maturation of the ova in the ovary. Further, the albumen does not attain as high a molybdenum concentration as the yolk, due to the lower concentration of molybdenum in the magnum as opposed to the ovary. The high concentration of molybdenum in the uterus suggests that the shell be considered in future studies of this nature.

Experiment IV - The Chemical Distribution of Molybdenum in Eggs from Hens Fed a Ration Containing 500 ppm of Molybdenum

The knowledge of the chemical distribution of a particular toxic substance in a biological system often provides clues that facilitate the elucidation of the mechanism of toxicity of that substance. The previous study indicated that the molybdenum transferred to the egg by the laying hen was not equally partitioned between the yolk and the albumen. These considerations prompted the more intensive study of the chemical distribution of egg molybdenum presented in the following section.

Experimental

The initial distribution studies involved standard fractionation techniques such as, ether extraction, trichloroacetic acid and ethanol precipitation, and dialysis. When it became apparent that about 25% of the total egg molybdenum was associated with the protein fraction, an effort was made to ascertain the mode of binding of molybdenum to protein.

To do this, a rat assay experiment was conducted which involved the feeding of the protein isolated from both control and molybdenum containing egg yolks. The experimental objectives were twofold. The first of these was to obtain information concerning the characterization of the molybdenum to protein binding. It was thought that if molybdenum were tightly bound to a specific amino acid of the protein molecule, a derangement in the metabolism of that amino acid or the protein could result and poorer growth would be observed in the rats consuming the molybdenum containing protein. The second purpose of the experiment arises from the observation that the toxic response of cattle to molybdenum differs depending on the form of the ingested molybdenum. Thus, the consumption of a forage containing 6-36 ppm of molybdenum by cattle results in a similar toxicosis (21) as the consumption of a feed containing 200-400 ppm of molybdenum added as the sodium molybdate salt (3). The production of a similar response in rats utilizing yolk protein bound molybdenum would simplify the study of this interesting effect.

With these objectives in mind, yolk protein was isolated in the following manner from both control and molybdenum containing eggs. Two kilograms of the frozen yolk material saved from Experiment II were slowly defrosted by means of warm tap water. Five hundred milliliters of ion free water were added and the resultant mixture was extracted with equal volumes of diethyl ether until the color of the extract was a pale yellow. Four liters of absolute ethanol were added to the residue from the ether extraction. The resultant precipitate was collected with suction on a Buchner funnel fitted with a coarse filter paper. The precipitate on the funnel was washed once with ether and then several times with methanol. The resultant protein preparation was dried at 70°C and stored in a refrigerator until use. With control yolk material, 265 g of the protein preparation were obtained which contained 1.07 ppm of molybdenum and 82.03% protein (N x 6.25) as determined by the Kjeldahl method. With molybdenum containing yolk material, these values were: 280 g obtained, containing 198.4 ppm of molybdenum and 80.72% protein. These preparations were used as the treatment modifications of the basal diet. The composition of the basal diet is listed in Table 8. In order to maintain the isonitrogenous status of diets formulated with the two protein preparations, the non-protein portion of the preparation was added at the expense of sucrose. Table 9 lists the dietary variables tested.

For the assay procedure, 20 male weanling albino rats of a disease resistant strain (DUB/DR) were purchased in four groups of five littermates[1]. The littermates were distributed so that each treatment group of four rats had equivalent sibling representation. The rats were housed individually in raised, stainless steel wire-floored cages and provided with food and tap water *ad libitum*. The animals were weighed weekly. At the termination of the

[1]Dublin Laboratory Animals, Dublin, Virginia.

Table 8.

Rat Basal Diet

Ingredient	Percent
Yolk protein	9.00
Sucrose (1)	82.50
Wesson oil	5.00
Mineral Mix (2)	2.50
Vitamin Mix (2)	1.00

(1) Included with the sucrose was the non-protein portion of the yolk protein preparation. This amounted to 2.03% in the case of the control yolk protein and 2.15% in the case of the molybdenum yolk protein.

(2) The composition of these components was described by Miller *et al*. (22).

Table 9.

The Dietary Variables Tested by the

Rat Assay Experiment

Group	Dietary Variable	Final Mo Concentration of Diet
A	9.00% control yolk protein	1.20
B	9.00% Mo yolk protein	20.96
C	9.00% Control yolk protein plus 20 ppm of Mo*	21.96
D	9.00% Mo yolk protein plus 100 ppm of Mo*	122.26
E	9.00% Control Yolk protein plus 120 ppm of Mo*	120.00

* Added as an aquaeous solution of sodium molybdate dihydrate.

four week experimental period, all rats were sacrificed by exsanguination and blood and liver samples were taken for molybdenum and copper analysis. Copper was determined by the carbamate method of the A.O.A.C. (2); molybdenum by the thiocyanate method of Evans _et al_. (11). The data presented in Table 10 on the characterization of the molybdenum protein was obtained in the following manner. A known quantity of molybdenum as sodium molybdate was added by trituration to the control protein preparation. This solution was then treated with either 10% trichloroacetic acid or absolute ethanol and the supernatant was analyzed for molybdenum. The amount of molybdenum precipitating with the protein was obtained by difference.

Other experimental procedures used in the characterization of the molybdenum protein will be outlined as alluded to in the following section.

Results and Discussion

The results of the initial fractionation studies are presented in Table 11. It can be seen that similar distributions of egg molybdenum were obtained with either trichloroacetic acid or ethanol as the protein precipitant. Further, although dialysis removed some of the molybdenum appearing in the protein fraction, the residual molybdenum in this fraction accounted for 25% of the total egg molybdenum. It is of interest to observe that only 3% of the egg molybdenum was present in the lipid fraction. This

Table 10.

The Coprecipitation of Molybdenum with Protein Precipitated by Either Trichloroacetic Acid or Ethanol

	TCA	Ethanol
Mo concentration of protein ($\mu g/ml$)	5.37 *	95.20 *
Mo concentration of precipitated protein ($\mu g/ml$)	4.50	84.00

* Average of three observations.

Table 11.

Distribution of the Molybdenum in Eggs from Hens Fed 500 ppm of Dietary Molybdenum

Trichloroacetic Acid Procedure			Ethyl Alcohol Procedure		
Egg Component*	Mo Content (μg)		Egg Component	Mo Content (μg)	
Albumen	98.9		Albumen	59.0	
Protein		72.4	Protein		9.6
Soluble		22.9	Protein (dialyzed)		6.8
			Soluble		51.8
Yolk	816.0		Yolk	816.0	
Ether Extract		39.0	Ether Extract		21.8
Protein		579.5	Protein		575.0
Soluble		228.0	Protein (dialyzed)		232.0
			Soluble		225.0

* The "protein" in each case refers to that fraction precipitating with the protein precipitant. The "soluble" refers to the supernatant from the protein precipitation.

would seem to exclude an involvement of molybdenum in phospholipid metabolism.

The weight gain and the blood and liver molybdenum and copper concentration data obtained from the rat assay study were subjected to analysis of variance. The analysis indicated that there was a highly significant effect of diet on weight gain, blood molybdenum and copper concentration and liver molybdenum concentration. Liver copper concentration was not affected by any of the dietary treatments. Duncan's multiple range test was used to separate the means of the significant effects noted.. A summary is presented in Table 12. Considering the data as a whole and especially the mean weight gain and the mean liver molybdenum concentration, there appears to be no indication of a differential response in the rat to the form of the dietary molybdenum.. Either of two alternate conclusions may apply to these data. First, the molybdenum bound to protein was in fact not very tightly bound either to a specific amino acid or to the protein molecule itself. On the other hand, the molybdenum could be tightly bound without appreciably affecting an observable response in the rat.

That the molybdenum was not tightly bound to the protein was later proven to be the case. The first indication of this was obtained from the observation that trituration of the molybdenum protein with a 10% solution of NaCl followed

Table 12.

Duncan's Multiple Range Test as Applied to the Variables Measured in the Rat Assay Experiment*

Dietary Group	Mean Weight Gain (g)	Blood (Mo) (μg/ml)	Blood (Cu) (μg/ml)	Liver (Mo) (ppm dry weight)
A	74.0^{a} ± 7.1	0.09^{a} ±0.04	0.66^{a} ±0.66	0.99^{a} ±0.15
B	65.3^{a} ± 5.7	1.96^{b} ±0.34	1.79^{ab}±0.38	2.93^{b} ±0.60
C	66.8^{a} ± 8.7	1.51^{b} ±1.30	1.11^{a} ±0.33	2.71^{b} ±0.43
D	47.5^{b} ±11.8	2.26^{b} ±0.83	2.29^{b} ±0.10	6.94^{c} ±0.42
E	43.5^{b} ±14.6	3.60^{c} ±1.00	1.93^{b} ±0.09	7.07^{c} ±1.25

* Means with the same superscript are not significantly different ($P \leq 0.05$). Each mean ±s.d. represents four observations.

by dialysis resulted in a loss of 95% of the molybdenum associated with the protein. Further studies of this nature indicated that both trituration and extensive dialysis were necessary to remove the molybdenum from the protein. Also, it was found that the removal of molybdenum was facilitated by alkaline conditions. Thus, 0.2N NaOH was more effective than either water or 0.2N HCl, and the water was more effective than the 0.2N HCl in removing the molybdenum from the protein. At this time, it was postulated that the molybdenum protein was an artefact of isolation. Thus, it was thought that molybdenum coprecipitated with the protein during the isolation procedure. This coprecipitate would be exceedingly difficult to separate by normal dialysis procedures and one would obtain a protein which would contain appreciable quantities of seemingly tightly bound molybdenum.

Evidence for this hypothesis is presented in Table 10. It can be seen that the molybdenum in a control protein preparation to which had been added a quantity of sodium molybdate does in fact appear in the protein fraction obtained by both trichloroacetic acid and ethanol precipitation. Although dialysis studies were not conducted on these protein fractions, it is assumed that they would behave similarly as the protein isolated from molybdenum containing egg yolks. As a broad conclusion from these studies, it is felt that molybdenum is not covalently bound to the protein molecule. However, ionic binding of a weak nature may exist.

Summary

Initial fractionation studies indicated that very little of the molybdenum in eggs from hens fed a diet containing molybdenum was present in the lipid fraction. Further, it appeared that 25% of the total egg molybdenum was associated with the protein fraction.

A rat assay of the molybdenum protein provided little evidence for a tight molybdenum to protein binding.

This finding was corroborated by further chemical studies which indicated that the molybdenum to protein binding may be an artefact of isolation. A combination of trituration and extensive dialysis served to remove the molybdenum from its association with the protein.

Experiment V - The Influence of Molybdenum Injected During Embryonic Development on The Subsequent Growth of the Hatched Chicks

The observation of a growth depression in chicks which had been injected with molybdenum during their embryonic development prompted the more extensive study presented in the following section (15).

Experimental

A 3x3x2 factorial experiment was designed which consisted of three dietary treatments and three injection treatments conducted with both sexes of chicks. The dietary treatments involved the feeding of a basal diet to which had been added 0, 500, and 1000 ppm of molybdenum. The injection treatments, made into the yolk sacs of embryos of ten days of incubation, were 3.00 mg of NaCl/egg, 0.50 mg of Mo/egg, and 1.00 mg of Mo/egg. Males and females were used in all treatment combinations.

Thus, 240 fertile DeKalb hybrid eggs purchased from Densmore Chick and Egg Farms, Inc. of Roanoke, Virginia, were incubated in the usual manner. After ten days of incubation, the survivors were separated into three equal groups, each of which being treated as previously mentioned. The details of the injection procedure have been described elsewhere (15). At term, the hatched chicks were maintained as separate injected groups and sexed. The chicks of each injected group were distributed among the dietary treatments so that six males and six females occurred in each block of the factorial design. The chicks were reared in the brooder facilities available at the V.P.I. Poultry Farm. They were weighed weekly throughout the four week experimental period. Food and water were provided *ad libitum*. The composition of the basal diet is listed in Table 13.

At the termination of the feeding period, all chicks were sacrificed by exsanguination. Blood was saved for copper and molybdenum analysis as well as hematocrit and hemoglobin determination. Hematocrits were determined by centrifuging 0.5 ml of blood in Wintrobe tubes. Hemoglobin was determined by an acid hematin method. Liver samples were saved for copper and molybdenum analysis.

Results and Discussion

The weight gain data from this experiment were subjected to analysis of variance. Table 14 summarizes the results of this analysis. An overall highly significant treatment effect on weight gain was indicated. Further, it can be seen that the dietary treatment could account for the major portion of the treatment effect on weight gain. The occurrence of a non-significant injection effect caused the rejection of the hypothesis that a growth depression occurs in chicks injected *in ovo* with molybdenum under the conditions of the experiment.

Table 13.

Basal Chick Diet

Ingredient	Percent
Sucrose	74
Casein	12
Gelatin	5
Salt Mix*	4
Wesson Oil	5

*As previously described. The vitamins were added directly to the basal diet in the following amounts per kilogram: thiamine - 0.6 mg, riboflavin - 0.9 mg, calcium pantothenate - 2.0 mg, niacin - 5.0 mg, pyridoxine - 0.8 mg, biotin - 0.03 mg, inositol - 100.0 mg, folic acid - 0.2 mg, B_{12} - 0.002 mg, A - 2000 I.U., D_3 - 200 I.C.U., α tocopherol acetate - 2.8 mg, menadione - 0.05 mg, choline - 200 mg.

Table 14.

Analysis of Variance of the Chick Weight Gain Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Among treatments	17	64,666.74	3,803.93	17.89**
Diets (D)	2	54,082.91	27,041.45	127.21**
Injections (I)	2	355.24	177.62	.84
Sex (S)	1	377.82	377.82	1.78
Interactions				
D x I	4	1,247.76	311.94	1.47
D x S	2	91.12	45.56	.21
I x S	2	224.57	112.28	.53
D x I x S	4	5,178.64	1,294.66	6.09**
Within Treatments	84	17,856.67	212.58	
Total	101	82,523.41		

** Significant at $P \leq 0.01$.

The occurrence of a significant third order interaction, although unexpected, can be neglected because if this interaction mean square were used to test the dietary effects, the differences observed would still be significant.

The data were pooled according to dietary treatment in order to permit separation of the mean weight gains by Duncan's multiple range test. The following separation was indicated at the $P \leq 0.01$ level:

Diet	1000 ppm Mo	500 ppm Mo	0 ppm Mo
Mean Weight Gain (g)	39.3	61.7	93.8

This distribution was expected, but it can be seen that the growth rate of all chicks, including the controls, were highly depressed. Since the basal diet contained 2.05 ppm of copper, it was thought that a true copper deficiency may have existed among all three dietary treatment groups.

More evidence for this supposed copper deficiency was obtained from the hemoglobin and hematocrit data. These data are summarized in Table 15. Considering only the data from the chicks fed the control diet (hemoglobin - 6.38 g/100 ml, hematocrit - 18.38%), an anemic condition can be postulated. Although no true positive controls were conducted, a comparison of these values with control values from the literature (hemoglobin - 7.72 g/100 ml, hematocrit - 31.20%) (18) would seem to indicate an anemic condition.

If this were a true copper deficiency and molybdenum does in fact interfere with copper metabolism, then in the

Table 15.

The Influence of Molybdenum Supplementation of Chick Diets on Blood Hemoglobin and Hematocrit Levels

Dietary Treatment	Hemoglobin (g/100 ml)	Hematocrit (%)
Control	6.38 ±0.75[1]	18.38 ±2.00[2]
500 ppm of Mo	5.79 ±0.97	17.43 ±2.21
1000 ppm of Mo	5.85 ±0.81	17.79 ±2.42

[1]Mean ±s.d. of 30 observations.
[2]Mean ±s.d. of 18 observations.

treatment groups supplemented with molybdenum, one would expect to obtain some accentuation of the copper deficiency anemia. Some indication of this was obtained from analysis of variance of the hemoglobin data from the three dietary treatment groups. This analysis indicated a significant effect of diet on the hemoglobin concentration of the blood ($P \leq 0.05$). However, referring again to Table 15, it can be seen that both levels of molybdenum supplementation resulted in a similar decrease in hemoglobin concentration. A reason for not obtaining a stoichiometric response in hemoglobin concentration with dietary molybdenum level will be presented later.

Analysis of variance of the packed cell volume data from the three treatment groups indicated that this parameter was not significantly affected by diet, although the means indicated a similar trend as that observed for the hemoglobin data. Small sample size as well as appreciable experimental error may serve to factor out a significant treatment effect on this variable.

The data obtained from the blood and liver molybdenum and copper analyses are of interest. These data are summarized in Table 16. Although statistical analyses were not conducted, certain trends are evident. There was very little response of the blood and liver molybdenum concentrations to the increased level of molybdenum in the diet. This may be attributed to a saturation of these tissues at the lower

Table 16.

The Influence of Molybdenum Supplementation of Chick Diets on Blood and Liver Molybdenum and Copper Concentration

Dietary Treatment	Blood[1]		Liver[2]	
	Mo	Cu	Mo	Cu
Control	1.45 ±0.83[3]	0.26 ±0.04	3.18 ± 1.20	8.44 ±1.42
500 ppm of Mo	28.68 ±6.18	0.26 ±0.09	35.94 ±10.50	9.21 ±1.38
1000 ppm of Mo	23.44 ±9.61	0.22 ±0.08	35.82 ±12.71	9.90 ±2.02

[1] Reported as μg/ml of whole blood. Each mean represents six observations of three pooled samples.

[2] Reported as ppm on a dry weight basis. Each mean represents 18 observations.

[3] Mean ±s.d.

level of supplementation with the consequence that they are not affected by any further increase in level of supplemented molybdenum. On this basis, the non-stoichiometry of the dietary effect on blood hemoglobin may be explained.

The blood and liver copper concentrations did not respond to molybdenum supplementation. This is in contrast to the data obtained with the growing rat which indicated that the copper concentration of these tissues doubled or tripled with molybdenum supplementation (22).

Summary

The observation of a growth depression in chicks which had been injected with molybdenum during their embryonic development was not corroborated.

A significant growth depression was obtained in chicks consuming diets containing molybdenum.

A copper deficiency may have existed in all chicks, in addition, some evidence for the involvement of molybdenum in copper metabolism was obtained.

Similar blood and liver concentrations of molybdenum were obtained when a diet containing either 500 or 1000 ppm of molybdenum was fed. The liver and blood concentration of copper was little affected by molybdenum supplementation.

Summary

In order to determine the feasibility of using the laying hen as a source of molybdenum containing eggs, a dose response experiment was conducted which involved the feeding of graded levels of molybdenum to White Rock laying hens. The feeding of 2000 ppm of molybdenum resulted in decreases in egg production and hen weight. Only egg production was decreased at 1000 ppm of molybdenum. The 500 ppm level of supplementation appeared to be most applicable to further studies in that little effect on either hen weight or egg production was noted. It was also observed that at this level the egg concentration of molybdenum plateaued at 16.4 ppm after 14 days of feeding the experimental ration.

An experiment designed to assess the toxicity of the molybdenum included in the egg by the laying hen to the developing chick embryo permitted the estimation of the $L.D._{100}$ to be about 17 ppm of molybdenum. This value agrees quite closely to the $L.D._{100}$ of yolk sac injected molybdenum. The embryonic death response observed in this experiment did not occur during any specific period of development. Succinoxidase assays conducted to study the death response indicated that there was no difference in succinoxidase activity of either the control or molybdenum treated embryos.

It was further noted that there was a decline in lethal response in embryos from hens fed the molybdenum containing

ration for periods in excess of three months. This decline was mediated by a decreased concentration of molybdenum in the eggs from these hens.

The mode of inclusion of molybdenum in the egg by the laying hen was studied. It appeared that the majority of the molybdenum is included during the maturation of the ova in the ovary. The high concentration of molybdenum observed in the uterus suggests that the shell be considered in future studies.

A chemical fractionation of the eggs from the hens fed the molybdenum containing diet indicated that very little of the molybdenum was present in the lipid fraction. Twenty-five percent of the total egg molybdenum was found to be associated with the protein fraction. A rat assay coupled with further chemical procedures indicated that the molybdenum to protein bonding was not of a covalent nature.

The observation of a growth depression in chicks which had been injected with molybdenum during their embryonic development was not corroborated by a more extensive study. Although this study was confounded by the possible existence of a copper deficiency, some evidence was obtained for the involvement of molybdenum in the copper metabolism of the chicks. It was surprising that no increase in the blood and liver molybdenum concentrations was observed when the diets containing either 500 or 1000 ppm of molybdenum were compared. The blood and liver copper concentrations were little affected by molybdenum supplementation.

Bibliography

1. Arthur, D., I. Motzok and H. D. Branion. Interaction of dietary copper and molybdenum in rations fed to poultry. Poultry Sci. 37: 1181 (1958)

2. Association of Official Agricultural Chemists Methods of Analysis. 8th Ed., p. 110 (1955)

3. Cox, D. H., G. K. Davis, R. L. Shirley and F. H. Jack. Influence of excess dietary molybdenum on rat and calf liver and heart enzymes. J. Nutrition 70: 63 (1960)

4. Davies, R. E., R. N. Burrough, B. L. Reid and J. R. Couch. Molybdenum interrelationships in chick nutrition. Poultry Sci. 37: 1196 (1958)

5. Davies, R. E., A. A. Kurnick, B. L. Reid and J. R. Couch. Molybdenum and sulfate interaction of the growing chick. Poultry Sci. 36: 1111 (1957)

6. Davies, R. E., B. L. Reid and J. R. Couch. Storage of molybdenum and copper in livers of chicks. Fed. Proc. 18: 522 (1959)

7. Davies, R. E., B. L. Reid, A. A. Kurnick and J. R. Couch. The effect of sulfate on molybdenum toxicity in the chick. J. Nutrition 70: 193 (1960)

8. DeRenzo, E. C. Studies on the nature of the xanthine oxidase factor. Ann. N. Y. Acad. Sci. 57: 905 (1954)

9. DeRenzo, E. C., E. Kaleita, P. G. Heyter, J. J. Oleson and B. L. Hutchings. Identification of the xanthine oxidase factor as molybdenum. Arch. Biochem. Biophys. 45: 247 (1953)

10. Duncan, D. B. Multiple range and multiple F tests. Biometrics 11: 1 (1956)

11. Evans, H. J., E. R. Purvis and F. E. Bear. Colorimetric determination of molybdenum by means of nitric and perchloric acids. Anal. Chem. 22: 1568 (1950)

12. Higgins, E. S., D. A. Richert and W. W. Westerfeld. Molybdenum deficiency and tungstate inhibition studies. J. Nutrition 59: 539 (1956)

13. Kratzer, F. H. Effect of dietary molybdenum upon chicks and poults. Proc. Soc. Exp. Biol. and Med. 80: 483 (1952)

14. Kurnick, A. A., B. L. Reid, R. N. Burrough, H. D. Stelzner and J. R. Couch. Effect of distillers dried solubles and molybdenum on the growing chick. Proc. Soc. Exp. Biol. and Med. 95: 353 (1957)

15. Lepore, P. D. The effects of injected molybdenum on the developing chick embryo. M. S. Thesis, Va. Polytech. Inst. (1960)

16. Lepore, P. D. and R. F. Miller. Molybdenum toxicity in the developing chick embryo. Fed. Proc. 20: 296 (1961)

17. Lepore, P. D., R. F. Miller and H. S. Siegel. Molybdenum toxicity in the developing chick embryo. Poultry Sci. 41: 1047 (1962)

18. Mahler, H. R., B. Mackler, D. E. Green and R. M. Bock. Studies on molybdoflavoproteins. III. Aldehyde oxidase: a molybdoflavoprotein. J. Biol. Chem. 210: 465 (1954)

19. Miller, E. C. and C. A. Denton. Feather depigmentation resulting from feeding molybdenum plus thiosulfate. Proc. Soc. Exp. Biol. and Med. 100: 179 (1959)

20. Miller, E. C. and C. A. Denton. Molybdenum - sulfate interrelationship in the growing chicks. Poultry Sci. 38: 910 (1959)

21. Miller, R. F. and R. W. Engel. Interrelations of copper, molybdenum and sulfate sulfur in nutrition. Fed. Proc. 19: 666 (1960)

22. Miller, R. F., N. O. Price and R. W. Engel. Added dietary inorganic sulfate and its effect upon rats fed molybdenum. J. Nutrition 60: 539 (1956).

23. Motzok, I., D. Arthur and H. D. Branion. Feeding of molybdenum to poultry. Poultry Sci. 36: 1144 (1957)

24. Potter, V. R. and W. C. Schneider. The assay of animal tissues for respiratory enzymes. J. Biol. Chem. 149: 217 (1943)

25. Reid, B. L., A. A. Kurnick, R. N. Burroughs, R. L. Svacha and J. R. Couch. Molybdenum in poult nutrition. Proc. Soc. Exp. Biol. and Med. 94: 737 (1957)

26. Reid, B. L., A. A. Kurnick, R. L. Svacha and J. R. Couch. The effect of molybdenum on chick and poult growth. Proc. Soc. Exp. Biol. and Med. 93: 245 (1956)

27. Ridgway, L. P. and D. A. Karnofsky. The effects of metals on the chick embryo: Toxicity and production of abnormalities in development. Ann. of N. Y. Acad. of Sci. 55: 203 (1952)

28. Siegel, P. B. Selection for breast angle at eight weeks of age. I. Poultry Sci. 41: 1177 (1962)

29. Sutherland, E. W., C. F. Cori, R. Haynes, and N. W. Olsen. Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa. J. Biol. Chem. 180: 825 (1949)

30. Teekell, R. A. and A. B. Watts. Tungsten supplementation of breeder hens. Poultry Sci. 38: 791 (1959)

31. Teekell, R. A. and A. B. Watts. Molybdenum supplementation of chick diets. Poultry Sci. 38: 1127 (1959)

32. Vohra, P. and F. H. Kratzer. The effect of dietary copper and molybdenum on turkey poults. Poultry Sci. 36: 1096 (1957)

33. Whiteside, C. H., T. M. Ferguson, B. L. Reid and J. R. Couch. Molybdenum, zinc, and unidentified factor sources in the diet of caged layers. Poultry Sci. 40: 313 (1961)

The vita has been removed from the scanned document

Abstract

In order to determine the feasibility of using the laying hen as a source of molybdenum containing eggs, a dose response experiment was conducted which involved the feeding of graded levels of molybdenum to White Rock laying hens. The feeding of 2000 ppm of molybdenum resulted in decreases in egg production and hen weight. Only egg production was decreased at 1000 ppm of molybdenum. The 500 ppm level of supplementation appeared to be most applicable to further studies in that little effect on either hen weight or egg production was noted. It was also observed that at this level the egg concentration of molybdenum plateaued at 16.4 ppm after 14 days of feeding the experimental ration.

An experiment designed to assess the toxicity of the molybdenum included in the egg by the laying hen to the developing chick embryo permitted the estimation of the $L.D._{100}$ to be about 17 ppm of molybdenum. This value agrees quite closely to the $L.D._{100}$ of yolk sac injected molybdenum. The embryonic death response observed in this experiment did not occur during any specific period of development. Succinoxidase assays conducted to study the death response indicated that there was no difference in succinoxidase.

It was further noted that there was a decline in lethal response in embryos from hens fed the molybdenum containing

ration for periods in excess of three months. This decline was mediated by a decreased concentration of molybdenum in the eggs from these hens.

The mode of inclusion of molybdenum in the egg by the laying hen was studied. It appeared that the majority of the molybdenum is included during the maturation of the ova in the ovary. The high concentration of molybdenum observed in the uterus suggests that the shell be considered in future studies.

A chemical fractionation of the eggs from the hens fed the molybdenum containing diet indicated that very little of the molybdenum was present in the lipid fraction. Twenty-five percent of the total egg molybdenum was found to be associated with the protein fraction. A rat assay coupled with further chemical procedures indicated that the molybdenum to protein bonding was not of a covalent nature.

The observation of a growth depression in chicks which had been injected with molybdenum during their embryonic development was not corroborated by a more extensive study. Although this study was confounded by the possible existence of a copper deficiency, some evidence was obtained for the involvement of molybdenum in the copper metabolism of the chicks. It was surprising that no increase in the blood and liver molybdenum concentrations was observed when the diets containing either 500 or 1000 ppm of molybdenum were compared. The blood and liver copper concentrations were little affected by molybdenum supplementation.