Manganese in the nutrition and metabolism of the pullet

1. Shell thickness and manganese content of eggs from birds given a diet of low or high manganese content

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1. The first two experiments were similar; pullets were given either a low-manganese diet, 6–7 μg/g, or a high-Mn diet containing an additional 50 μgMn/g; shell thickness as measured by weight per unit area was obtained for the first four and the last four eggs produced during a short laying period and the Mn contents of the first two and last two eggs were determined.

2. Shell thickness was significantly reduced by the low-Mn diet in Expt 1, but not in Expt 2. In both experiments there was an increase in thickness from the first four to the last four shells produced.

3. The Mn content of eggs from birds given the low-Mn diet was significantly lower in both experiments than that from birds given the high-Mn diet. In Expt 1 there was an increase in Mn content from the first to the last eggs laid, but no corresponding change occurred in Expt 2.

4. The differences between the results of the two experiments are discussed; they were probably due to differences in the time for which the experimental diets were given before laying began.

5. In Expt 3 pullets were given the high-Mn or low-Mn diet from about 4 weeks before laying began or the high-Mn diet to the point-of-lay and then the low-Mn diet. Shell thickness was determined in batches of six eggs from each bird at intervals during a 6- to 7-month laying period.

6. Shell thickness was depressed by the low-Mn diet in birds given this diet 4 weeks before laying but not in those given the low-Mn diet from the point-of-lay.

7. In Expt 4 shell thickness was determined on the first thirty eggs laid by four pullets given the high-Mn diet and four given the low-Mn diet from about 4 weeks before starting to lay. The difference between shells from birds given the high-Mn and low-Mn diets just failed to reach significance and there was no significant increase or decrease in the series of thirty shells from each bird, studied in successive groups of six shells.

The first report indicating an effect of dietary manganese on egg-shell formation was that of Caskey & Norris (1938): at the end of this brief abstract the authors stated that the breaking strength and ash content of egg-shells increased with the quantity of Mn in the diet. Lyons (1939) gave a more detailed account of the effects of dietary Mn on egg-shells: those from birds given a diet containing 7 μg Mn/g were rough and had translucent areas when candled, whereas those from birds given the same diet plus 50 μg Mn/g were normal in appearance. When the diets were reversed the shells changed accordingly and, when some birds from each group were given the low-Mn diet plus 20 μg Mn/g, the shells were good in general, but some birds produced poor shells. When a change of diet was made the shells were affected relatively quickly, the full effect usually being recorded in about 2 weeks, and there was no evidence that the effects of level of dietary Mn on shell formation were any different at the end of a 6-month laying period than they had been at the start. These results were all obtained

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by grading the shells on appearance but, in addition, a small number of determinations of shell strength and ash content were made that confirmed the statements of Caskey & Norris (1938) given earlier, and also showed a close relationship between values given by visual grading and ash content.

Gutowska & Parkhurst (1942), using basal diets of higher Mn content than that of Lyons, 17 or 24 μg/g, found a significantly lower shell strength than with the same diet supplemented with about 50 μg Mn/g. No differences in egg production, fertility or hatchability were observed and shell texture of eggs from all birds seemed generally satisfactory on candling and there were few soft-shelled or cracked eggs.

There are no other reports on the effects of dietary Mn on shell formation except for a recent abstract (Mathers & Hill, 1965) that arose partly from the present study, in which the principal interest was in the shell and in particular the effect of a low-Mn diet on shell thickness as measured by weight per unit area. The Mn contents of some of the eggs used for shell measurement were also determined.

EXPERIMENTAL

Expt 1. Eighteen Rhode Island Red × Light Sussex (RIR × LS) pullets 19 weeks of age were divided randomly into two groups, one being given a low-Mn and the other a high-Mn diet. The basal low-Mn diet containing 6 μg Mn/g was similar to that described earlier (Hill, 1965); its percentage composition was: maize meal 66, barley meal 10, white fish meal 6, dried separated milk 6, dried brewer's yeast 6, calcium carbonate 3, tricalcium phosphate 2·5 and salt plus vitamins 0·5. A supplement of manganese carbonate to provide a further 50 μg/g was added to give the high-Mn diet. Each group of birds remained throughout the experiment on the diet allocated.

Shell thickness, as measured by the weight per unit area method described by Taylor (1965) was determined for the first four shells produced by each bird and for the last four before the birds were killed after about 2·5 months of egg production. The contents of the first two and the last two eggs were dried, fat-extracted, ignited and pooled for Mn determination by the standard permanganate method (Association of Official Agricultural Chemists, 1945). The total number of eggs laid per bird and the number of soft-shelled eggs were recorded.

Expt 2. This was similar to Expt 1 except that the birds were the reverse cross (LS × RIR) and were about a week older when the experiment began. Also the period of egg production was shorter—about 1·5 months.

Expt 3. Twenty-one light hybrid pullets aged 18 weeks were divided randomly into three groups, each of seven birds (LL, HH and HL). Group LL was given the low-Mn diet containing 6–7 μg Mn/g from 18 weeks of age throughout the experiment, group HH was given the same diet with $MnCO_3$ added to give a further 100 μg/g throughout, and group HL was given the high-Mn diet to point-of-lay and then the low-Mn diet. The experiment was terminated after a 6- to 7-month period of egg production, and the birds were then killed for determination of tissue Mn content; the results are reported by Mathers & Hill (1968).

The number of soft-shelled eggs was recorded and shell thickness, measured as weight per unit area, was determined for the first six shelled eggs from each bird and

for further batches of six eggs from each bird at approximately 2, 4 and 6 months after the start of laying.

Expt 4. Four pullets were given the high-Mn diet (106–107 μg/g) and four the low-Mn diet (6–7 μg/g) from about 4 weeks before laying began and weight per unit area was determined for shells of the first thirty shelled eggs produced by each bird. The number of days taken to produce the thirty eggs was recorded.

RESULTS

Expt 1. Most of the birds started to lay 3–4 weeks after the introduction of the low-Mn and high-Mn diets. The mean number of eggs laid per bird was similar for each group; the mean for the low-Mn treatment was 53 of which a mean of 2·7 eggs per bird was soft-shelled. The corresponding numbers for the high-Mn group were 56 and 3·0 respectively. Although there were slightly more soft-shelled eggs from high-Mn than from low-Mn birds over the whole period, for the first ten eggs laid by each bird the reverse was true (low-Mn 2·4, high-Mn 1·7) and, perhaps more important, all low-Mn birds laid at least one soft-shelled egg whereas only five of the nine high-Mn birds laid any soft-shelled eggs.

Mean values of weight per unit area of shells and Mn contents of eggs for each group at the beginning and end of the laying period are given in Table 1. There were significant effects on shell thickness of level of dietary Mn and stage of egg production, shells from low-Mn birds having a lower weight per unit area than those from high-Mn birds, and birds on both treatments giving shells of greater weight per unit area at the end than at the start of the laying period. However, the difference between shells from low-Mn and high-Mn birds, though significant for the first four shells, just failed to reach significance for the last four, giving a significant interaction between level of dietary Mn and stage of egg production.

Some shells produced by birds given the low-Mn diet had an uneven, rough-looking surface, and translucent areas when viewed with transmitted light (Pl. 1). Fewer abnormal shells were noted at the end than at the start of the experiment.

The Mn contents of eggs (Table 1) showed a significant effect of time in the egg-laying period as well as the expected effect of level of dietary Mn, the Mn content of eggs laid on each diet at the start of egg laying being lower than those produced after 2·5 months of egg production. There was also a significant interaction between diet and time, the increase in Mn content with time being greater for high-Mn than for low-Mn birds. These differences were more striking for total Mn content than for concentration in dry fat-free material, an expression of an increase in egg size with age.

Expt 2. Egg production began the day after the introduction of the experimental diets and the average period of feeding before the start of egg-laying was 1–2 weeks. During the 1·5 months egg-laying period the number of eggs laid by the low-Mn birds was 32/bird and by the high-Mn birds 35/bird; of these 6·3 and 7·6/bird respectively were soft-shelled. This unusually high proportion of soft-shelled eggs was probably associated with the precocious sexual development of the birds caused by exposure to days of increasing length during rearing: there was no difference between treatments in numbers of soft-shelled eggs.

Table 1. *Expt 1: mean weight per unit area of shells and manganese content of eggs from birds given a diet of low or high Mn content*

Diet	No. of birds	Wt per unit area (means for four shells/bird) (mg/cm²)			Mn content of eggs					
					Total/egg (μg)			Concentration (μg/g dry fat-free egg)		
		First shells	Shells after 2·5 months	Mean for dietary treatment	First two eggs	Two eggs after 2·5 months	Mean for dietary treatment	First two eggs	Two eggs after 2·5 months	Mean for dietary treatment
Low-Mn	9*	56·6	67·2	61·9	2·8	5·5	4·2	0·55	0·76	0·66
High-Mn	9*	66·3	71·5	68·9	7·0	13·0	10·0	1·19	1·80	1·50
Mean for stage of production		61·4	69·4	—	4·9	9·2	—	0·87	1·28	—
SE of difference between means		Diets ±3·03 Stages ±1·48 Diets within a stage ±2·85 Stages within a diet ±1·39			Diets ±0·499 Stages ±0·502 Diets within a stage ±0·704 Stages within a diet ±0·708			Diets ±0·078 Stages ±0·078 Diets within a stage ±0·110 Stages within a diet ±0·110		
Statistical significance of difference, *P*		Diets < 0·05 Stages < 0·001 Diets × stages < 0·05			Diets < 0·001 Stages < 0·001 Diets × stages < 0·01			Diets < 0·001 Stages < 0·001 Diets × stages < 0·05		

*For Mn content of eggs, there were complete results for eight birds per dietary group.

Table 2. *Expt 2: mean weight per unit area of shells and manganese content of eggs from birds given a diet of low or high Mn content*

Diet	No. of birds	Wt per unit area (means for four shells/bird) (mg/cm²)			Mn content of eggs					
					Total/egg (μg)			Concentration (μg/g dry fat-free egg)		
		First shells	Shells after 1·5 months	Mean for dietary treatment	First two eggs	Two eggs after 1·5 months	Mean for dietary treatment	First two eggs	Two eggs after 1·5 months	Mean for dietary treatment
Low-Mn	9*	59·9	65·5	62·7	6·0	3·8	4·9	1·35	0·69	1·02
High-Mn	9*	61·3	67·2	64·2	9·6	10·5	10·0	1·81	1·88	1·84
Mean for stage of production		60·6	66·4	—	7·8	7·2	—	1·58	1·28	—
SE of difference between means		Diets ±2·55 Stages ±1·76 Diets within a stage ±3·60 Stages within a diet ±2·48			Diets ±0·847 Stages ±0·778 Diets within a stage ±1·194 Stages within a diet ±1·097			Diets ±0·170 Stages ±0·154 Diets within a stage ±0·240 Stages within a diet ±0·217		
Statistical significance of difference, *P*		Diets NS Stages < 0·01 Diets × stages NS			Diets < 0·001 Stages NS Diets × stages NS			Diets < 0·001 Stages NS Diets × stages < 0·05		

NS, not significant.

* For Mn content of eggs, there were complete results for eight birds per dietary group.

Mean weights per unit area and Mn contents of eggs at the start and end of the egg-laying period are given in Table 2. There was a significant increase in weight per unit area with time as in Expt 1, but in contrast, no significant effect of level of Mn and no interaction were observed. As in Expt 1 the shells of some eggs from low-Mn birds had rough-surfaced and translucent areas. No record of numbers of such shells was kept but there were probably fewer than in Expt 1.

The effect of level of dietary Mn on the Mn content of eggs was significant, and the interaction between level of dietary Mn and stage of egg production given by a decrease in Mn content of eggs from low-Mn birds during the period of egg production was significant for concentration of Mn but just failed to reach significance for weight of Mn per egg. The relatively small difference between the Mn contents of eggs from low-Mn and high-Mn birds at the start of laying was no doubt a reflection of the short time the low-Mn diet was given before laying began.

Table 3. *Expt 3: mean weight per unit area of shells at intervals during a 6- to 7-month laying period from birds given a low-Mn (LL) or high-Mn (HH) diet or changed from high to low at the point-of-lay (HL)*

Dietary treatment	No. of birds	Wt per unit area (means for six shells/bird) (mg/cm²) First shells	Shells after 2 months	Shells after 4 months	Shells after 6 months	Mean for dietary treatment
HH	7	77·6	77·9	74·7	73·3	75·9
LL	7	70·0	72·7	68·7	68·0	69·8
HL	7	79·5	77·6	75·0	74·8	76·7
Mean for stage of production		75·7	76·1	72·8	72·0	—
SE of difference between means		Diets ±1·94 Stages ±1·04 Diets within a stage ±3·88 Stages within a diet ±1·80				
Statistical significance of difference, *P*		Diets < 0·01 Stages < 0·001 Diets × stages NS				

NS, not significant.

Expt 3. Egg production began approximately 7 weeks after the start of the experiment and mean percentage egg production during the 6–7 months laying period was 80 for group LL, 81 for HH and 83 for HL. The total number of soft-shelled eggs produced was only 18 and most were produced by birds in group LL, and in the first 2 or 3 weeks after the start of lay. Five birds of group LL gave at least one soft-shelled egg while only one of HH and two of HL gave a soft-shelled egg.

Mean weights per unit area of shells produced at 2-monthly intervals for each treatment are given in Table 3. There was a significant effect of dietary treatment on shell thickness: shells of group LL birds were thinner than those from birds of groups HH and HL. The effect of time was also significant, shells becoming thinner between 2 and 4 months after the start of laying. This decrease in thickness with time was greater for groups HH and HL than for group LL and in consequence the effect of dietary treatment was smaller at the end than at the beginning of the 6-month laying period, but the interaction between dietary treatment and time was not significant. The

thickness of shells of birds changed from the high-Mn to the low-Mn diet at the point-of-lay (group HL) was remarkably similar to that of shells from birds given the high-Mn diet throughout the experiment (group HH), there being no tendency for the low-Mn diet given from the point-of-lay through the 6-month laying period to depress shell thickness.

Shells with rough and translucent areas were produced, but only in the early stages of egg production by birds of group LL.

Expt 4. Successive eggs laid by individual birds varied in shell thickness fairly widely and, to reveal possible changes in thickness in the first thirty eggs produced by individual birds of this experiment, means of each six successive eggs were analysed statistically. The dietary treatment means for each group of eggs with appropriate standard errors and the mean number of days taken to produce the thirty eggs are given in Table 4.

Table 4. *Expt* 4: *mean values for weight per unit area of the first thirty shells produced by birds given a diet of low or high manganese content*

Diet	No. of birds	Mean no. of days for thirty eggs	Wt per unit area (mean for six consecutive shells) (mg/cm²) 1–6	7–12	13–18	19–24	25–30	Mean
Low-Mn	4	37·5	68·9	70·0	66·9	71·6	70·4	69·6
High-Mn	4	36·5	77·0	75·8	74·1	73·8	77·2	75·6
Mean			73·0	72·9	70·5	72·7	73·8	—
SE of difference between means			Diet ±2·49		Groups of eggs ±1·75			
Statistical significance of difference, *P*			Diet < 0·10 > 0·05		Groups of eggs NS			

NS, not significant.

No uniform trend towards increasing or decreasing shell thickness from the first six to the last six of the series of thirty eggs was found either from means for dietary groups or from over-all means. However, the shells formed during the middle part of the laying period studied tended to be poorer than those formed at the beginning or end: this was seen most clearly in the over-all means. Egg-laying was slightly less uniform in low-Mn than in high-Mn birds, there being more days on which two eggs were laid by low-Mn than by high-Mn birds and more days on which no egg was laid by low-Mn than by high-Mn birds, but when these irregularities were taken into account in a covariance analysis there was very little evidence that either had any effect on the general picture of shell thickness in the period studied. The effect of level of dietary Mn on shell thickness approached significance at the 5% level and there was no interaction between diet and group of eggs.

DISCUSSION

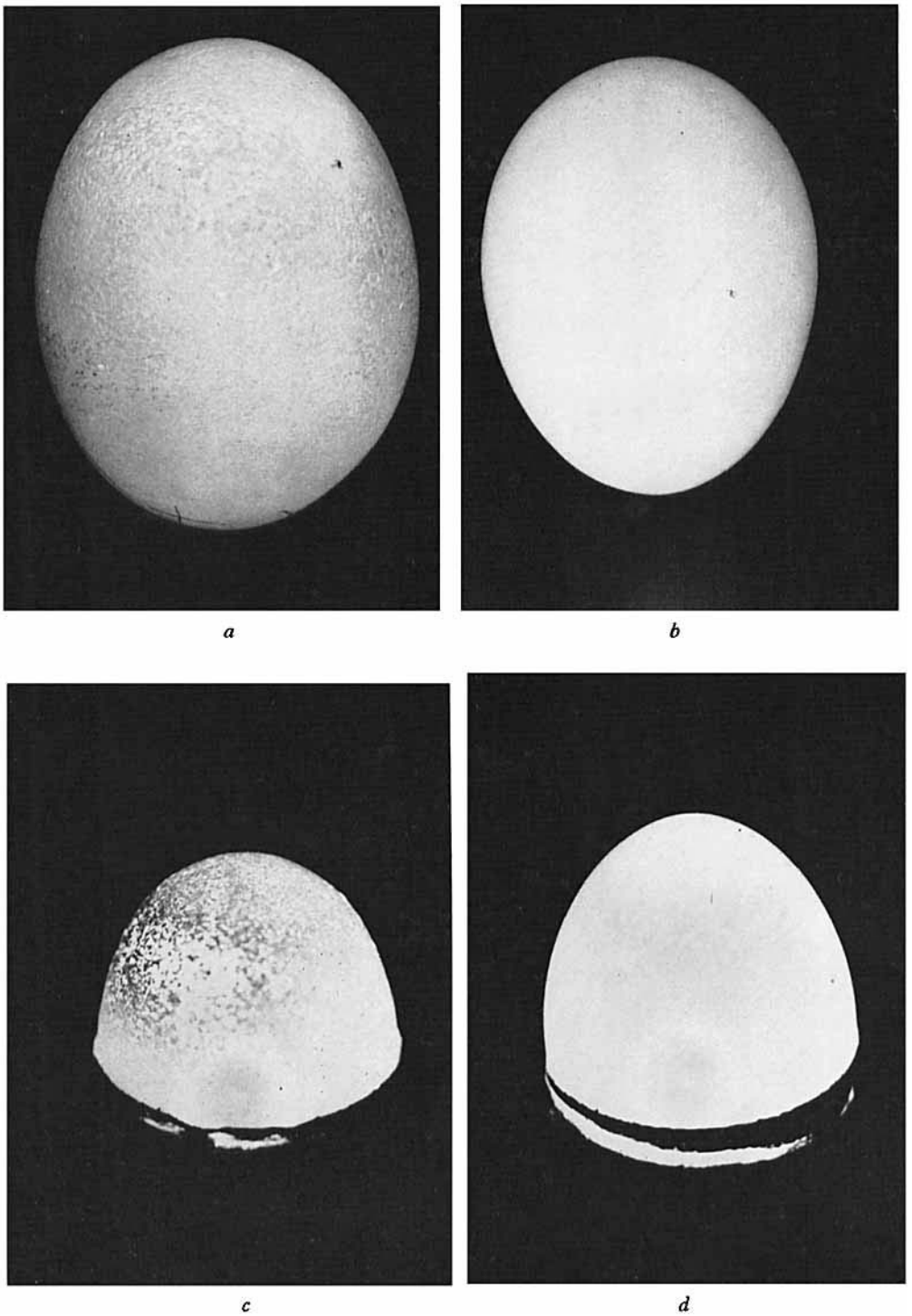
In each experiment some shells of abnormal appearance, similar to those described by Lyons (1939), were produced. Though the nature of the abnormalities resembled closely those observed by Lyons, there were differences in the circumstances in which they occurred. In the present study no abnormal shells were observed from birds

given the low-Mn diet from the point-of-lay, but only from those given the low-Mn diet from before sexual maturity (Expt 3). However, in the experiments of Lyons the low-Mn diet that gave the abnormalities was introduced after the birds had come into lay, and shell texture responded within 2 weeks to changes in diet made during egg production. The low-Mn diet used by Lyons contained a higher level of phosphorus (1·7%) than that used in the present study (1·0%) and may account for the differences between their experiments and ours.

Results for shell thickness, measured by weight per unit area, were strikingly different in some respects between Expts 1 and 2, there being a significant effect of level of dietary Mn in the former but not in the latter. The chief differences between the two experiments were the nature of the breeding cross, RIR × LS in the first and LS × RIR in the second, and the period before egg laying for which the low-Mn diet was given. It was thought unlikely that the difference in response to dietary Mn was attributable to reversing the cross, but rather that the effect of the low-Mn diet on egg-shell formation occurred only when the diet was given for several weeks before egg laying began, as in Expt 1. In Expt 3 this possibility was investigated and found to be true, at least for the particular low-Mn diet used in this study. The total absence of any effect on shell thickness when the low-Mn diet was introduced at the point-of-lay and given for 6–7 months, was a surprising feature of the results. It appears that when the low-Mn diet used in this study was given during the period of sexual maturation an essential physiological component of the process of shell formation developed at a slower rate than normal, but once development of this component was completed shell formation proceeded normally.

The significant increase in shell thickness that occurred in approximately 2 months of egg production in the first two experiments did not occur in Expt 3, nor in the more detailed study of a 5-week laying period, Expt 4. Samples of feed were taken for Mn determination at intervals during each experiment and no marked changes in Mn content were found, so that the increase in shell thickness with time in Expts 1 and 2 could not be explained by an increase in Mn intake. The difference between Expts 1 and 2 on the one hand and 3 and 4 on the other may be related to the genetical make-up of the birds; those of Expts 1 and 2 were crosses from RIR and LS parents whereas those of Expts 3 and 4 were by birds derived wholely or largely from White Leghorn stock. It is of interest to note that in general, shell thickness was greater in Expts 3 and 4 than in 1 and 2. The decrease in shell thickness that occurred in Expt 3 could not be compared with results from Expts 1, 2 and 4, these experiments having terminated before the decrease in Expt 3 occurred.

The Mn content of the eggs of Expt 1 can be interpreted with greater confidence than that in Expt 2 because in the latter a diet of high but unspecified Mn content was given until only a very short time before egg laying began, whereas in Expt 1 the experimental diets of known Mn content were given for several weeks before the first egg was laid. The chief point of interest in the results for Mn content of the eggs in Expt 1 is the increase that occurred during the 2·5 months of egg production. As the Mn contents of the diets were constant, it is suggested that an increase in retention of dietary Mn occurred during the laying period.



a *b*

c *d*

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EXPLANATION OF PLATE

Shells of eggs laid by pullets given a diet of low or high manganese content. (*a*) Rough surface, low-Mn diet (reflected light). (*b*) Smooth surface, high-Mn diet (reflected light). (*c*) Translucent areas, low-Mn diet (transmitted light). (*d*) Uniformly opaque shell, high-Mn diet (transmitted light).

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