The zinc requirements of broiler chicks and turkey poults fed on purified diets

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- 1. Chicks and turkey poults were fed for 3 weeks on low-zinc diets, prepared from purified ingredients, supplemented with zinc oxide at graded levels.
- 2. Birds of both species given the unsupplemented basal diets grew poorly, with high mortality rates. All had severe hyperkeratosis but bone development was normal. Only when birds received diets with low concentrations of added Zn were leg abnormalities observed.
- 3. Zn requirements were assessed visually from dose-response graphs. The chick required 18 mg Zn/kg diet for maximal live weight and 24 mg Zn/kg for maximal Zn concentration in blood serum. The responses of tibial Zn and net retention of Zn did not reach plateaux within the range of dietary Zn concentrations studied. The turkey poult's Zn requirement for maximal live weight was 25 and 28-29 mg/kg for net retention of Zn and for maximal concentration of Zn in blood plasma and in the tibia; 41 mg Zn/kg diet was required for maximal Zn in blood serum.
 - 4. Liver Zn was not correlated with dietary Zn in either species.

The requirement of the chick for zinc has been estimated to be 15–20 mg/kg diet when the protein in the purified diet was egg albumen or a mixture of casein and gelatin (Moeller & Scott, 1958; Pensack et al. 1958; Zeigler et al. 1961) and about 30 mg/kg when the diet contained isolated soya-bean protein (Roberson & Schaible, 1958; Zeigler et al. 1961). The higher requirement when soya-bean protein was used has been attributed to the impairment of Zn availability by phytic acid (O'Dell & Savage, 1960).

The estimate of 64 mg/kg for the Zn requirement of the turkey poult has been based only on experiments in which the dietary protein was isolated soya-bean protein (Kratzer et al. 1958; Sullivan, 1961).

The main criteria used in assessing the Zn requirements of chicks and turkey poults have been the attainment of maximal live weight and normal bone, skin and feather development.

The object of the present study was to assess the Zn requirement of chicks and turkey poults when both were fed on a purified diet containing egg albumen as the protein source. Spray-dried albumen powder, which has been commonly used in purified diets for poultry, has been shown to be unsuitable for feeding to turkeys and chicks because of its high sodium content (Dewar & Siller, 1971; Siller et al. 1972). Thus the low-Na albumen prepared by the method of Dewar & Siller (1971) was used.

In addition to live weight and bone, skin and feather development, net retention of Zn and the concentrations of Zn in liver, blood and tibia were examined as criteria for assessing the Zn requirement.

EXPERIMENTAL

Diets

The main dietary ingredients were maize starch (Corn Products Ltd, Manchester), cellulose (Bayer Chemicals Ltd, Surrey) and low-Na egg albumen, prepared by the method of Dewar & Siller (1971) from spray-dried albumen (Henningsen, New York). Totanin (Toten

Ingredients	Chick	Turkey
 Starch	500	441
Low-sodium egg albumen	270	310
Cellulose	100	100
Maize oil	50	60
Totanin*	20	20
CaCO ₃	10	16
CaHPO ₄ . 2H ₂ O	35	35
KCl .	5	8
Mineral mix†	5	5
Vitamin mix‡	5	5

Table 1. Composition (g/kg) of basal low-zinc chick and turkey diets

Cellulosefabrik, Norway), an ammonium lignosulphonate, was included to facilitate pelleting of the diet by the method of Dewar (1969).

The compositions of the low-Zn basal diets for chicks and turkeys are given in Table 1. The chick diet was found by analysis to contain 7 mg Zn/kg and the turkey diet 6 mg/kg.

Birds and husbandry

The chicks were from a PRC broiler strain derived from a commerical stock. The turkey poults were from a commericial strain (T6 British United Turkey).

Cages were of Perspex $(560 \times 360 \times 330 \text{ mm})$ with food and water troughs of the same material. The cage floors were of PVC-covered wire mesh in a Perspex frame. The ambient temperature was maintained at 25° with additional heating supplied by Eltex dull-emitter brooder lamps, each lamp being suspended centrally above a group of four cages.

As-hatched chicks or poults were wing-banded, weighed and assigned randomly to the cages. Pelleted diets and ion-free water were available ad lib. Birds were weighed and food consumption recorded regularly. After 7 d, when yolk sac absorption was complete, droppings were collected on Perspex trays until the end of the experiment. To minimize contamination of the droppings, spilled food was removed regularly from the trays throughout the day. Droppings were dried to constant weight in Pyrex dishes at 100° in a forced-draught oven.

Sample preparation and analysis

Samples of blood were obtained by cardiac puncture at the end of Expts 1 and 3. Serum and plasma were deproteinized with trichloroacetic acid (100 g/l; Hoch & Vallee, 1949) before Zn analysis. Livers were freeze-dried, weighed and ground to a fine powder. Bones were dissected from articular cartilage, extracted with diethyl ether to remove fat and then dried at 100° to constant weight.

Following a double ashing procedure at 450°, solid samples were extracted with 6 M-hydrochloric acid. Zn analysis was carried out on a Varian AA5 atomic absorption spectrophotometer.

^{*} Lignosulphonate binder, see text.

[†] Supplied (/kg diet): MgCO₃. Mg(OH)₂. 3H₂O 2·6 g, FeSO₄. 7H₂O 300 mg, MnSO₄. 4H₂O 500 mg, CuSO₄. 5H₂O 20 mg, CoSO₄. 4H₂O 1 mg, KIO₃ 2 mg, Na₂MoO₄. 2H₂O 9 mg, Na₂SeO₄. 10H₂O 0·46 mg.

[‡] Supplied (/kg diet): aneurine 40 mg, nicotinic acid 80 mg, riboflavin 30 mg, pantothenic acid 40 mg, folic acid 6 mg, cyanocobalamin 0.5 mg, pyridoxine 12 mg, choline 3000 mg, menaphthone 3 mg, biotin 3 mg, α -tocopherol 100 mg, retinol 600 μ g, cholecalciferol 12.5 μ g.

Table 2. Expt 1. The effects of feeding diets with different zinc concentrations to chicks (Mean values with their standard errors)

Group		1	2	3	4	5	6
Total Zn in diet (mg/kg)		7	11	15	19	23	27
Mortality (no. of birds)	_	8	0	0	0	0	0
Severity of skin lesions at 21 d*		4.5	2.8	0	0	0	0
Severity of leg abnormalities at 21 d*		0	4-2	0	0	0	0
Live-wt at 21 d (g)	Mean	82.6	189-9	314-2	382.0	378-4	387.9
	SE	3.5	8.0	13.2	13.1	11.5	9.6
Food consumption (g)	Mean	60.5	266-3	418.5	506.5	519.5	526-3
	SE	4.2	3.3	14-7	14.6	32.6	20-7
Live-wt gain: food consumption	Mean		0.518	0.647	0.679	0.697	0.675
	SE		0.004	0.006	0.035	0.025	0.020
Net retention of Zn (mg Zn/kg	Mean	_	23-4	24.3	29.1	31.0	32.7
live-wt gain 8-21 d)	SE		0.7	0.9	2.3	1.0	2.0
Net retention of Zn	Mean		86.3	86-4	86.0	81.1	73.8
(% Zn eaten 8-21 d)	SE		1.2	1.0	1.1	1.0	1.6
Zn in:							
Liver (mg/kg dry matter)	Mean	95.9	81.0	81-1	72.6	84.7	90.6
(2, 0 , ,	SE	9.3	3.4	5-2	3.4	3.5	5.0
Tibia (mg/kg dried	Mean	15.2	43.2	67.0	131-1	155.6	193.3
defatted tibia)	SE	4.8	2.6	1.8	1.3	5.3	5-1
Blood serum (mg/1)	Mean	0.96	0.95	1-16	1.68	2.21	2.24
()	SE	0.01	0.01	0.01	0.01	0.01	0.01

^{*} Scored according to a subjective assessment in which 0 was normal and 5 most severe.

Table 3. Expt 2. The effects of feeding diets with different zinc concentrations to turkey poults

(Mean values with their standard errors)

Group Total Zn in diet (mg/kg)		1 6	2 21	3 36	4 51	5 66
Mortality (no. of birds)		15	1	0	1	
Severity of skin lesions at 21 d*	_	4.5	3.2	ŏ	Ô	ŏ
Severity of leg abnormalities at 21 d*	_	0	4.2	0	Ō	Ō
Live-wt at 21 d (g)	Mean	66-1†	311.0	410-4	383-2	390-9
(6)	SE		12.0	17.0	13.7	18.6
Food consumption (g)	Mean	40.5	361-5	467-0	452.5	429-5
	SE	2.8	17.8	21.3	7.0	12.7
Live-wt gain: food consumption	Mean		0.741	0.772	0.749	0.803
	SE		0.023	0.044	0.033	0.016
Net retention of Zn (mg/kg	Mean		24.3	35.7	37.6	31.8
live-wt gain 8-21 d)	SE		0.9	2.2	1.6	2.2
Net retention of Zn (% Zn	Mean		84.7	71.1	50.3	38.5
eaten 8-21 d)	SE		1.2	1.8	2.1	2.7

^{*} Scored according to a subjective assessment in which 0 was normal and 5 most severe.

[†] Live weight of one bird surviving at 21 d.

Table 4. Expt 3. The effects of feeding diets with different zinc concentrations to turkey poults

(Mean values with their standard errors)

Group		1	2	3	4	5	6
Total Zn in diet (mg/kg)		16	21	26	31	36	41
Mortality (no. of birds)		3	4	4	1	1	2
Severity of skin lesions at 21 d*	_	0	0	0.	0	0	0
Severity of leg abnormalities at 21 d*		2.2	0	0	0	0	0
Live-wt at 21 d (g)	Mean	298.3	374.5	415.0	412.5	418.6	414.2
	SE	8.4	11.8	15.2	11.8	16.6	16.7
Food consumption (g)	Mean	315.0	417-2	468.8	457-7	461.7	439.8
	SE	9.5	22.5	7-8	10.9	20.3	16.2
Live-weight gain: food	Mean	0.783	0.772	0.773	0.781	0.799	0.772
consumption	SE	0.035	0.104	0.020	0.015	0.030	0.030
Net retention of Zn (mg/kg	Mean	19·5	24.7	30.0	32.3	32.7	34-1
live-wt gain 8-21 d)	SE	1.1	0.8	0.6	0.5	1.7	1.1
Net retention of Zn (% Zn	Mean	88.9	88.5	86.3	82.5	73.5	66.6
eaten 8-21 d)	SE	0.6	1.3	1.3	1.1	2.3	1.7
Zn in:							
Liver (mg/kg dry matter)	Mean	76 ·1	69.8	73.7	80.9	74.8	92.1
	SE	2.9	2.1	1.8	3.3	2.5	3.2
Tibia (mg/kg dried	Mean	53.6	102.7	150.6	178.7	195.6	187.0
defatted tibia)	SE	2.8	4.6	4.7	4.2	7 ·7	4.1
Blood serum (mg/l)	Mean	1.30	2.02	2.66	2.93	3.17	3.21
	SE	0.12	0.10	0.11	0.11	0.11	0.10
Blood plasma (mg/l)	Mean	0.94	1.55	2.18	2.58	2.55	2.67
• • • •	SE	0.12	0.09	0.10	0.10	0.10	0.10

^{*} Scored according to a subjective assessment in which 0 was normal and 5 most severe.

Experimental procedure

There were three experiments, each lasting 21 d. At the end of each experiment all birds were scored subjectively for severity of skin and leg lesions using a scale on which 0 was normal and 5 most severe. The sex of each bird was determined post mortem by internal examination.

Expt 1. There were six diets: the basal low-Zn chick diet (Zn content 7 mg/kg) and five diets prepared from it by adding zinc oxide to give Zn concentrations of 11, 15, 19, 23 and 27 mg/kg. Each diet was supplied to four cages; each cage contained four as-hatched chicks.

Net retention of Zn was calculated from 8 to 21 d following total collection of the droppings. Live weight and Zn concentrations in liver, tibia and blood serum at 21 d were also measured.

Expt 2. There were five diets: the basal low-Zn turkey diet (Zn content 6 mg/kg) and four diets prepared from it by adding ZnO to give Zn concentrations of 21, 36, 51 and 66 mg/kg. Each diet was supplied to four cages; each cage contained four as-hatched poults.

Only net retention of Zn from 8 to 21 d and live weight at 21 d were measured.

Expt 3. Six diets containing 16, 21, 26, 31, 36 and 41 mg Zn/kg were made by adding ZnO to the low-Zn turkey diet. Each diet was supplied to six cages; each cage contained five as-hatched poults.

Net retention of Zn from 8 to 21 d, live weight and the concentration of Zn in liver, tibia and blood serum and plasma were measured at 21 d.

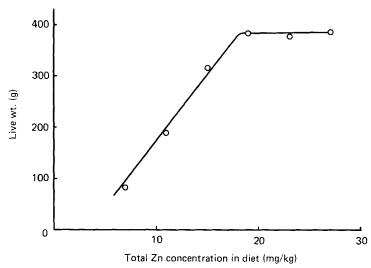


Fig. 1. Expt 1. The effect of feeding diets containing various amounts of zinc (mg/kg) on the 21 d live weight of chicks.

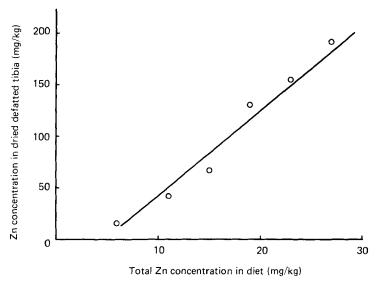


Fig. 2. Expt 1. The effect of feeding diets containing various amounts of zinc (mg/kg) on the concentration of Zn in dried, defatted tibias (mg/kg) from chicks.

RESULTS

The results of Expts 1, 2, and 3 are summarized in Tables 2, 3 and 4 respectively. Because of high mortality rates, food conversion efficiency was not calculated for birds in group 1 in Expts 1 and 2. The amounts of droppings voided by birds in these groups were very small and estimation of net retention was not considered practicable.

It was found by analysis of variance that there were no sex effects in any of the experiments.



Fig. 3. Expt 1. The effect of feeding diets containing various amounts of zinc (mg/kg) on the net retention of Zn (mg/kg live-weight gain) from 8 to 21 d by chicks.

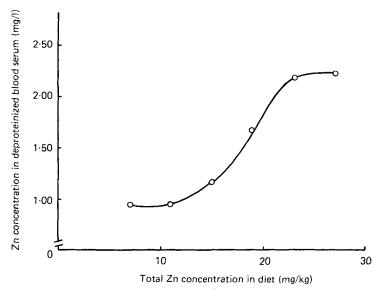


Fig. 4. Expt 1. The effect of feeding diets containing various amounts of zinc (mg/kg) on the concentration of Zn in deproteinized blood serum (mg/l) from chicks.

The response curves in Figs, 1-9 were drawn by eye. An exception was the response of tibial Zn to dietary Zn in Expt 1 (Fig. 2, p. 471) for which a linear regression seemed appropriate. Response curves for liver Zn in Expts 1 and 3 were not drawn as there appeared to be no obvious trend with dietary Zn.

Expt 1. After several days the chicks in group 1, receiving the unsupplemented low-Zn diet, became anorexic and thereafter showed little or no increase in weight. At 7 d, hyperkeratotic lesions were noticed on the feet and on the skin around the beaks of several birds. Eventually all birds became severely affected by these lesions. Abnormal feather

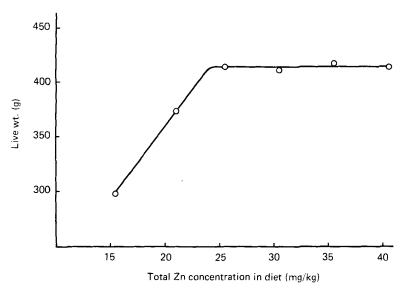


Fig. 5. Expt 3. The effect of feeding diets containing various amounts of zinc (mg/kg) on the 21 d live weight (g) of turkey poults.

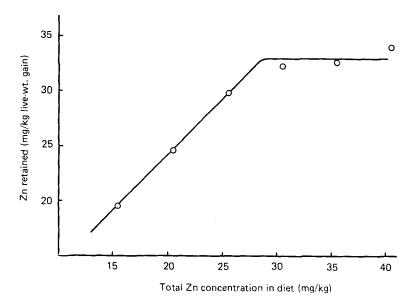


Fig. 6. Expt 3. The effect of feeding diets containing various amounts of zinc (mg/kg) on the net retention of Zn (mg Zn/kg live-weight gain) from 8 to 21 d by turkey poults.

development, similar to that described by Young et al. (1958) was seen in all birds. Eight birds died between the 13th and 21st days.

Chicks in group 2 showed similar but less severe lesions. In addition, all birds developed leg abnormalities. Affected limbs appeared to be shorter and thicker than usual, with swollen hocks. Various limb deformities and abnormal limb torsion were common. Despite the severity of the lesions there was no mortality.

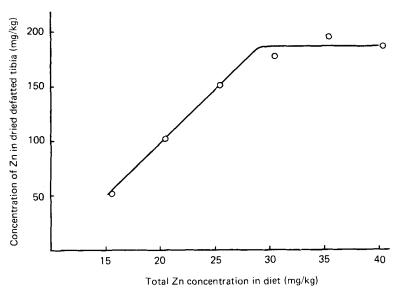


Fig. 7. Expt 3. The effect of feeding diets containing various amounts of zinc (mg/kg) on the concentration of Zn in dried, defatted tibias (mg/kg) from turkey poults.

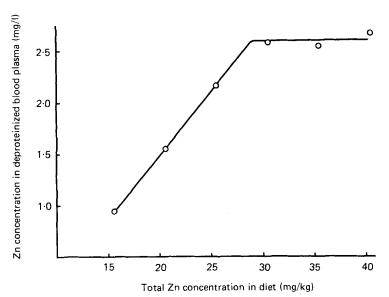


Fig. 8. Expt 3. The effects of feeding diets containing various amounts of zinc (mg/kg) on the concentration of Zn in deproteinized blood plasma (mg/l) from turkey poults.

Birds in groups 3, 4, 5 and 6 grew without loss and showed none of the skin, feather and leg abnormalities observed in groups 1 and 2.

The requirement of Zn using 21-d live weight as the criterion was estimated from Fig. 1 to be 18 mg/kg. The response of tibial Zn (Fig. 2) differed from the other responses in being nearly linear (r^2 0.98). As neither it nor the response of net retention of Zn (Fig. 3) reached a plateau, no assessments of requirement were made. The curve for serum Zn

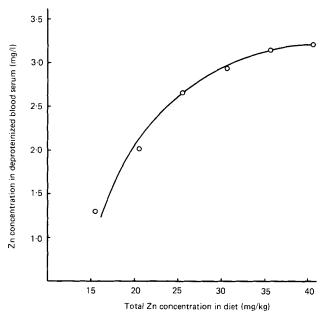


Fig. 9. Expt 3. The effect of feeding diets containing various amounts of zinc (mg/kg) on the concentration of Zn in deproteinized blood serum (mg/l) from turkey poults.

(Fig. 4) was sigmoid; from it the requirement for maximal Zn concentration in blood serum was estimated to be 24 mg/kg.

Expt 2. Turkey poults in group 1 showed a similar pattern of food intake and growth to that of chicks in group 1 in Expt 1. There were also similarities in the development of Zn deficiency. All birds suffered from skin lesions and were poorly feathered, but they showed no evidence of leg deformity. Poults died from the 9th day until, at the end of the experiment, only one bird survived.

In group 2, skin lesions were less severe and feathering was better than in group 1; however, all birds were severely affected by leg abnormalities. Only one bird in the group died.

Birds in groups 3, 4 and 5 showed none of the lesions associated with Zn deficiency. There was one death (in group 4) as a result of severe pecking.

It was inferred from the values for live weight and net retention (Table 3) that the requirements lay between 20 and 40 mg Zn/kg. The dietary Zn levels in Expt 3 were chosen to cover this range.

Expt 3. There were four cases of moderately-severe leg abnormalities in group 1, but no evidence of skin lesions or poor feathering. There were three deaths.

Feathering was normal and there were no skin or bone lesions in groups 2–6. Several early deaths were associated with infected yolk sacs. Two of the birds that died in group 3 were severely pecked after having their legs caught in the cage floor.

From the live weight response curve (Fig. 5) the requirement for Zn was estimated to be 25 mg/kg. Based on net retention (Fig. 6), tibial Zn (Fig. 7) and plasma Zn (Fig. 8) the requirement was 28-29 mg/kg. An estimate of requirement was not made from the response curve for serum Zn (Fig. 9) as no plateau was reached within the range of dietary Zn concentrations examined.

DISCUSSION

The feeding of the low-Zn basal diets resulted in severe Zn deficiencies in both chicks and turkey poults. More detailed descriptions of the signs of Zn deficiency in similarly-depleted birds have been given elsewhere (Wight & Dewar, 1978, 1979; Gentle et al. 1981; Dewar et al. 1982). In both species the mortality rate was higher and the impairment of growth greater than has been reported for deficient birds in other Zn-requirement studies. Another important difference from the results of other workers was that neither chicks nor poults in the most severely depleted groups had leg abnormalities. It was only when a small amount of Zn was added to the diet, as in group 2 in Expts 1 and 2, that bone lesions developed. These lesions were grossly similar to those described in Zn-deficient chicks by O'Dell et al. (1958) and by Young et al. (1958) and in turkey poults by Sullivan (1961).

An interesting feature of Expt 3 was that none of the birds in group 2 showed leg deformities although their diet had the same level of Zn as that in the diet eaten by the birds in group 2 in Expt 2. In the latter group all birds were severely affected by leg bone abnormalities. Food consumption and live weights in group 2 in Expt 3 were higher than those in group 2 in Expt 2, but the values for net retention were similar.

The Zn requirement of 18 mg/kg assessed from the chick live-weight values in Expt 1 is close to the estimates of 15 mg/kg (Zeigler et al. 1961) and 20 mg/kg (Pensack et al. 1958) when the diet contained casein and gelatin. Moeller & Scott (1958) found that 10 mg added Zn/kg diet was sufficient for maximal growth in chicks fed on a diet which contained albumen. The Zn content of their basal diet is not stated but by analogy with similar diets in other experiments, might be expected to have contained 5-8 mg Zn/kg.

The Zn requirement for maximal live weight for the turkey poult was estimated to be 24 mg/kg and for net retention of Zn and tibial and plasma Zn 29 mg/kg. However, 41 mg Zn/kg was required for maximal Zn concentration in blood serum. These estimates are much lower than the 64 mg/kg reported by Kratzer et al. (1958) and Sullivan (1961) to be necessary for maximal live weight and normal bone development in the turkey poult. In contrast to the present study the Zn requirement for maximal live weight was lower than that for maximal bone development in both these reports.

On the basis of the evidence of net retention from Expts 2 and 3 it seems that the turkey has little ability to store Zn once its immediate requirements are met. In keeping with this observation, maximal values for tibial and plasma Zn were attained at the same dietary Zn level as was net retention. The situation in the chick was less clear, with the response curves for net retention and tibial Zn failing to reach plateaux within the range of dietary Zn levels studied. Weigand & Kirchgessner (1979) have suggested that the net retention of trace minerals should form the basis for calculating requirements and practical allowances. Their calculated value of 27 mg Zn retained/kg weight gain is low compared with values in the present study. More research, with larger numbers of birds of different ages and with a wider range of dietary Zn concentrations, is needed to test the suitability of net retention as an index of Zn deficiency in the chick.

No experiments have been reported in which the Zn requirements of chicks and turkey poults have been studied at the same time on similar diets. The most direct comparison that can be made, using the results of Roberson & Schaible (1958) and Zeigler et al. (1961) for chicks and of Kratzer et al. (1958) and Sullivan (1961) for turkey poults, points to the turkey having a requirement approximately twice that of the chick. The results of the present study suggest that the requirements are much closer to each other. A possible explanation for the turkey's apparently high requirement when the diet contains soya-bean protein is that the turkey is in some way affected more than the chick by the presence of dietary phytate. A complicating factor in interpreting the role of phytate in such interferences is

that isolated soya-bean proteins from different commercial sources can vary in the extent to which they affect Zn utilization (Vohra & Kratzer, 1967).

The presence of feedingstuffs of plant origin, such as cereals and soya-bean meal, which contain phytate, must be taken into account when applying the results of basic requirement studies to the formulation of practical diets. A further difficulty which must be considered is that, in small-scale experiments, birds do not always grow to their genetic potential. This may be because the purified diet is poorly consumed; for example, when a group of turkey poults, of the same strain as those used in this study, was reared under similar conditions on a commercial diet their mean live weight at 21 d was 543 g (W. A. Dewar, unpublished results) compared with the highest mean live weight of 418 g in Expt 2. Poor growth may also result from sub-optimal environmental conditions. Whitehead & Bannister (1980) have put forward evidence that, when chicks were restricted in their growth by the environment, the requirement for biotin was reduced to a level necessary to support the reduced growth rate. They suggest that the requirement for any nutrient is likely to depend on the level of performance.

Factors such as these may help to explain the report by Tortuero & Brennes (1976) that more than 80 mg Zn/kg was necessary for maximal performance in broilers kept under practical conditions and fed on diets based on maize and soya-bean meal.

REFERENCES

Dewar, W. A. (1969). Journal of Agricultural Science, Cambridge 92, 325-326.

Dewar, W. A., Sibbald, I. R. & Wight, P. A. L. (1982). British Poultry Science 22, 129-134.

Dewar, W. A. & Siller, W. G. (1971). British Poultry Science, 12, 535-543.

Gentle, M. J., Dewar, W. A. & Wight, P. A. L. (1981). British Poultry Science 22, 265-273.

Hoch, F. L. & Vallee, B. L. (1949). Journal of Biological Chemistry 181, 295-306.

Kratzer, F. H., Vohra, P., Allred, J. B. & Davis, P. N. (1958). Proceedings of the Society for Experimental Biology and Medicine 98, 205-207.

Moeller, M. W. & Scott, H. M. (1958). Poultry Science 37, 1227-1228.

O'Dell, B. L., Newberne, P. M. & Savage, J. B. (1958). Journal of Nutrition 65, 513-518.

O'Dell, B. L. & Savage, J. E. (1960). Proceedings of the Society for Experimental Biology and Medicine 103, 304-306.

Pensack, J. M., Henson, J. N. & Bogdonoff, P. D. (1958). Poultry Science 37, 1232-1233.

Roberson, R. H. & Schaible, P. J. (1958). Poultry Science 37, 1321-1323.

Siller, W. G., Dewar, W. A. & Whitehead, C. C. (1972). Journal of Pathology 107, 191-197.

Sullivan, T. W. (1961). Poultry Science 40, 334-340.

Tortuero, F. & Brennes, A. (1976). Advances en Alimentacion y Mejora Animal 18, 139-143.

Vohra, P. & Kratzer, F. W. (1967). Poultry Science 46, 1016-1019.

Weigand, E. & Kirchgessner, M. (1979). In World's Poultry Science Association 2nd European Symposium, pp. 64-71 [C. A. Kan and P. C. M. Simons, editors]. Beekbergen: The Netherlands.

Whitehead, C.C. & Bannister, D. W. (1980). British Journal of Nutrition 43, 541-549.

Wight, P. A. L. & Dewar, W. A. (1978). In *Trace Element Metabolism in Man and Animals-3*, pp. 215-217 [M. Kirchgessner, editor]. Freising-Weihenstephan: Technische Universität München.

Wight, P. A. L. & Dewar, W. A. (1979). Avian Pathology 8, 437-451.

Young, R. J., Edwards, H. M. Jr & Gillis, M. B. (1958). Poultry Science 37, 1100-1107.

Zeigler, T. R., Leach, R. M., Norris, L. C. & Scott, M. L. (1961). Poultry Science 40, 1584-1593.