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Effects of short- and long-term feeding of zinc oxide-supplemented diets on the mature, female domestic fowl with special reference to tissue mineral content

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1. In Expt 1, the effects on laying hens of diets supplemented with zinc oxide providing up to 20 g added zinc/kg were compared. In Expt 2 the diets contained up to 6 g added Zn/kg.

2. In both experiments, food intake, body-weight, egg number, and liver, oviduct and ovary weights/kg body-weight were significantly reduced by added ZnO; gizzard weight/kg body-weight was significantly increased. In Expt 2, pancreas weight was significantly reduced by added ZnO.

3. Liver, kidney and pancreatic Zn and iron concentrations were significantly elevated in both experiments.

4. In both experiments, liver, kidneys and pancreatic copper concentrations gave quadratic responses to added ZnO.

Zinc toxicity is not normally a problem encountered with domestic animals. It has been established that pigs (Cox & Hale, 1962), rats (Magee & Spahr, 1964), cattle (Miller *et al.* 1965) and sheep (Ott *et al.* 1966*a*) exhibit considerable tolerance to high dietary concentrations of Zn compounds. Several reports have confirmed that the performance of broiler fowl is not adversely affected by the dietary inclusion of zinc oxide to provide up to 1 g Zn/kg diet (Roberson & Schaible, 1960; Johnson *et al.* 1962; Kincaid *et al.* 1976*b*).

Although the supplementation of diets with excessive levels of Zn compounds has been suggested as a method for the induction of a resting phase for laying hens (Creger & Scott, 1977; Shippee *et al.* 1979), there is relatively little information available concerning the effects of offering adult hens diets containing high levels of Zn compounds. In the mature, female domestic fowl, Hermayer *et al.* (1977) and Palafox & Ho-A (1980) demonstrated that the dietary inclusion of Zn compounds, at levels providing up to 10 and 20 g Zn/kg diet respectively, caused a severe depression in food consumption, egg production and body-weight. More recently, Gentle *et al.* (1982) identified a threshold level of ZnO addition at or above 6 g Zn/kg diet which produced a rapid reduction in the food intake of adult hens.

Dietary addition of excessive amounts of Zn compounds to diets being consumed by broiler chicks has been shown to cause a marked increase in liver Zn concentration (Johnson *et al.* 1962; Kincaid *et al.* 1976*b*) and in the pancreatic tissue Zn concentration of cockerels (Eltohamy *et al.* 1980). Studies on the specific effects of high dietary levels of ZnO on the storage of tissue Zn in mature domestic fowl have been very limited and preliminary investigations, both short- and long-term, into the effects of high dietary levels of ZnO on both the performance and tissue mineral accumulation of laying hens were therefore initiated.

MATERIALS AND METHODS

Expt 1

Seventy-two Hisex laying hens (thirty-six white; thirty-six brown), 40 weeks of age, were placed in galvanized iron cages fitted with individual feeder troughs and nipple drinkers.

Table 1. *Composition (g/kg) and analysis of the control diet as fed*

Composition (g/kg)	
Ground maize	600·0
Ground wheat	74·2
Soya-bean-meal extract (431 g CP/kg)	187·5
White fish meal (623 g CP/kg)	26·1
Dried grass meal (158 g CP/kg)	25·0
Limestone flour	71·9
Dicalcium phosphate	9·8
Sodium chloride	3·0
Vitamin–mineral supplement*	2·5
Total	1000·0
Analysis as fed (/kg)	
Dry matter (g)	982·0
Metabolizable energy (MJ)†	11·0
CP (g)	159·9
Diethyl ether extract (g)	25·1
Ash (g)	109·0
Calcium (g)	29·5
Phosphorus (g)	5·8
Zinc (mg)	56·0
Iron (mg)	361·0
Copper (mg)	6·0

CP, crude protein (nitrogen × 6·25).

* Provided (/kg diet): 1·76 mg retinol, 35 μg cholecalciferol, 2·9 mg riboflavin, 4·9 μg cyanocobalamin, 5·8 mg α-tocopherol, 0·7 mg menadione sodium bisulphite, 10 mg nicotinic acid, 5·8 mg calcium D-pantothenate, 200 mg choline chloride, 1·5 mg potassium iodide, 14·4 mg Fe, 0·1 mg selenium, 2·0 mg cobalt, 7·2 mg manganese, Zn-free and Cu-free.

† Calculated.

The poultry house was unheated, the maximum and minimum temperatures being 14° and 4° respectively. A lighting regimen of 17 h light – 7 h dark was maintained during the experiment. The birds were randomly allocated to one of six treatment groups, each comprising six birds of each hybrid strain. The diets, offered *ad lib.* for 3 weeks, were the control diet (Table 1), and this diet supplemented with 4, 8, 12, 16 and 20 g Zn as finely-powdered ZnO/kg.

The birds were weighed initially and subsequently body-weight and food consumption recorded weekly and egg number daily. The eggs laid on 2 d/week were weighed. The birds given diets with 20 g added Zn/kg were removed from the experiment at 10 d because food intake was severely depressed. After 3 weeks the birds were killed by decapitation.

The gizzard, oviduct and ovary were weighed, and liver, kidneys, pancreas, spleen and adrenals weighed and retained for dry matter, Zn, iron and copper determinations. Tissue mineral concentrations were determined by atomic absorption spectrophotometry subsequent to dry ashing and solution in hydrochloric acid. Liver lipid concentrations were assayed on dried samples (Folch *et al.* 1957).

Before weighing, the gizzards were cut open, washed and the koilin layer examined to determine the gross effects of dietary treatment. Tissue samples were removed from the previously-weighed liver and kidneys of two birds taken at random from the control treatment, and of two birds from the highest dietary Zn treatment. The samples were then preserved in buffered neutral formalin (100 ml/l) and examined histopathologically.

The results were subjected to analysis of variance, log transformations being carried out for those variables which exhibited variance heterogeneity.

Expt 2

Ninety-eight Hisex laying hens (forty-nine white; forty-nine brown), 40 weeks of age, were housed as in Expt 1. The maximum and minimum temperatures were 18° and 2° respectively. They were randomly allocated to one of seven treatment groups each containing seven birds of each hybrid strain. Diets, offered *ad lib.* for five consecutive 28 d periods, consisted of the control diet, as used in Expt 1 (Table 1), and this diet supplemented with 1, 2, 3, 4, 5 and 6 g Zn as ZnO/kg.

Body-weights were determined initially and at the end of each period. Food consumption was measured for each period. Daily records were made of egg production and eggs were weighed on 2 d/week. At the end of the experiment four birds of each strain were randomly selected from each treatment and killed by decapitation.

Specific organs were removed, weighed and analysed for mineral concentrations. Liver lipid concentration was determined as for Expt 1. Liver, kidney and gizzard samples were selected at random from two control birds and from two receiving the highest level of added dietary ZnO. They were preserved in buffered neutral formalin and subsequently given an histopathological examination. Statistical analyses were carried out as for Expt 1.

RESULTS

Expt 1

No birds died during the experiment although this would possibly not have been the case if the birds on the highest level of ZnO supplementation had not been removed from the experiment after 10 d. Feather loss, although not excessive, was apparent for the birds offered diets with 12–20 g added Zn/kg; breed differences were not evident. Damage of the gizzard lining, including erosion and rupture, was observed in approximately 20% of the birds given 16 g supplemental dietary Zn as ZnO/kg.

Mean weekly food and total Zn intakes, initial and final body-weights and weekly egg production are given in Table 2. Mean weekly food intake, body-weights and egg numbers were highly significantly depressed by the dietary inclusion of ZnO. The maximum ZnO intake occurred at the 4 and 8 g/kg level of addition for the brown and white strains respectively.

During weeks 2 and 3 food consumption was significantly lower for the brown than for the white birds. In the final week the food intakes of the white and brown hens offered the diet providing 16 g Zn/kg were reduced to 9 and 2% of their respective controls.

Egg production had ceased by the end of the 1st week for birds on the 12–20 g Zn/kg treatments and by the end of the 2nd week for hens on the 8 g Zn/kg treatment. Even 4 g added Zn/kg diet induced an almost complete pause in lay for both breeds by the 2nd week.

Tissue weights/kg body-weight together with liver lipid values are given in Table 3. The tissues investigated, with the exception of kidneys and pancreas, showed a response to dietary treatment. The liver fresh weight and the oviduct and ovary weights showed decreasing responses, the mean weights for the oviduct and ovary being minimum at the 8 g added Zn/kg diet. Gizzard weight/kg body-weight showed an increasing response to ZnO supplementation. The liver lipid concentrations and total contents were significantly decreased by added ZnO.

Results of the tissue Zn, Fe and Cu analyses are presented in Tables 4–6 respectively. Dietary inclusion of ZnO significantly increased both Zn concentrations and total contents (Table 4) of all the tissues examined except in the case of the adrenal glands.

The liver Fe concentrations and contents (Table 5) were increased up to 400 and 200% respectively of the control group by dietary treatment while for kidneys the Fe concentration

Table 2. *Expt 1. Mean weekly food and total zinc intakes, body-weights and egg numbers of mature, female domestic fowl given control and zinc oxide-supplemented diets*

	Breed†	Week of experi-ment	Level of added Zn (g/kg): 0	4	8	12	16	20	SEM: Diet	SEM: Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Food intake (g)	1	1	685	297	163	77	41	18	—	26·9	Overall	NS	***	NS
	2	1	767	295	98	28	33	8			Linear		***	NS
											Quadratic		***	*
	1	2	705	438	213	110	46	—	—	38·2	Overall	*	***	NS
	2	2	791	324	87	27	18	—			Linear		***	NS
											Quadratic		***	**
	1	3	769	340	241	148	70	—	—	32·1	Overall	*	***	NS
	2	3	817	273	121	68	20	—			Linear		***	NS
											Quadratic		***	*
Zn intake‡ (mg)	1	1–3	120	3443	3999	3819	2344	—	—	(0·0693)	Overall	***	***	***
			(2·080)	(3·537)	(3·602)	(3·582)	(3·370)				Linear		***	***
	2	1–3	131	2917	1837	995	1069	—			Quadratic		***	NS
			(2·119)	(3·465)	(3·264)	(2·998)	(3·029)							
Body-wt (kg)	1+2	0	1·93	1·92	1·92	1·93	1·93	1·92	0·015	—	Overall	***	NS	NS
											Linear		NS	NS
											Quadratic		NS	NS
	1+2	1	1·89	1·72	1·61	1·59	1·55	1·55	0·020	—	Overall	***	***	NS
											Linear		***	NS
											Quadratic		***	NS
	1+2	2	1·86	1·72	1·51	1·47	1·43	—	0·024	—	Overall	***	***	NS
											Linear		***	NS
											Quadratic		***	NS
	1	3	1·55	1·34	1·19	1·08	1·03	—	—	0.034	Overall	***	***	*
	2	3	2·19	1·93	1·61	1·62	1·56	—			Linear		***	NS
											Quadratic		***	*
Egg no.	1+2	1	6·5	4·5	3·8	3·3	3·4	2·9	0·20	—	Overall	***	***	NS
											Linear		***	NS
											Quadratic		***	NS
	1	2	5·7	0·2	0·0	0·0	0·0	—	—	0·18	Overall	NS	***	NS
	2	2	6·5	0·2	0·2	0·0	0·0	—			Linear		***	*
											Quadratic		***	NS
	1+2	3	6·0	0·6	0·0	0·0	0·0	—	0.19	—	Overall	NS	***	NS
											Linear		***	NS
											Quadratic		***	NS

NS, not significant.
* $P<0·05$. *** $P<0·01$.
† Breed 1, Hisex white; breed 2, Hisex brown.
‡ Mean values are antilogs of the mean of the log transformations which are given in parentheses.

Table 3. *Expt 1. Mean fresh weights of some tissues expressed as g/kg body-weight together with the liver lipid concentration (g/kg dry matter) and total liver lipid content (g) of mature, female domestic fowl given control and zinc oxide-supplemented diets*

	Breed†	Level of added Zn (g/kg) 0	4	8	12	16	SEM Diet	SEM Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Tissue weights												
Liver	1	21·1	25·0	17·8	20·5	19·8	—	1·01	Overall	***	***	*
	2	18·0	17·4	13·6	12·4	12·2			Linear		***	*
									Quadratic		NS	NS
Spleen	1+2	0·71	1·13	1·20	1·08	0·91	0·091	—	Overall	NS	**	NS
									Linear		NS	NS
									Quadratic		***	NS
Adrenals	1+2	0·056	0·093	0·077	0·103	0·096	0·0076	—	Overall	***	***	NS
									Linear		***	NS
									Quadratic		NS	NS
Ovary	1	26·5	17·2	1·9	2·1	2·6	—	2·21	Overall	**	***	*
	2	16·8	6·9	1·5	2·0	1·7			Linear		***	**
									Quadratic		***	NS
Oviduct	1	34·3	16·8	4·3	4·9	4·5	—	1·87	Overall	**	***	*
	2	24·5	9·6	3·9	4·0	4·1			Linear		***	**
									Quadratic		***	NS
Gizzard	1+2	11·8	17·6	20·6	23·7	23·0	0·79	—	Overall	**	***	NS
									Linear		***	NS
									Quadratic		***	NS
Liver lipid												
Concentration	1+2	298	144	158	137	144	16·1	—	Overall	NS	***	NS
									Linear		***	NS
									Quadratic		***	NS
Total content	1+2	3·72	1·39	1·02	0·84	0·82	0·263	—	Overall	NS	***	NS
									Linear		***	NS
									Quadratic		***	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Breed 1, Hisex white: breed 2, Hisex brown.

Table 4. *Expt 1. Mean zinc concentrations (μg/g dry matter) and total contents (μg) of some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets*

	Breed†	Level of added Zn (g/kg) 0	4	8	12	16	SEM Diet	SEM Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Zn concentrations												
Liver‡	1	99	1641	1718	1888	1644	—	(0·0462)	Overall	***	***	**
		(1·995)	(3·215)	(3·235)	(3·276)	(3·216)			Linear		***	**
	2	97	1750	1114	1000	1086			Quadratic		***	NS
		(1·989)	(3·243)	(3·047)	(3·000)	(3·036)						
Kidneys‡	1	117	402	750	953	897	—	(0·0504)	Overall	**	***	NS
		(2·068)	(2·604)	(2·875)	(2·979)	(2·953)			Linear		***	*
	2	131	450	535	638	615			Quadratic		***	NS
		(2·119)	(2·563)	(2·728)	(2·805)	(2·789)						
Pancreas‡	1+2	95	4899	4560	3707	4560	—	—	Overall	NS	***	NS
		(1·976)	(3·690)	(3·659)	(3·569)	(3·659)	(0·0414)	—	Linear		***	NS
									Quadratic		***	NS
Spleen	1	85	100	116	129	112	—	5·3	Overall	***	***	•
	2	90	95	95	99	96			Linear		***	**
									Quadratic		**	NS
Adrenals	1	43	43	37	45	47	—	4·6	Overall	*	NS	NS
	2	45	41	36	29	34			Linear		NS	*
									Quadratic		NS	NS
Total Zn contents												
Liver‡	1	1057	15070	10140	11940	9397	—	(0·0507)	Overall	**	***	**
		(3·024)	(4·178)	(4·006)	(4·077)	(3·973)			Linear		***	***
	2	1216	16480	7621	5902	6124			Quadratic		***	NS
		(3·085)	(4·217)	(3·882)	(3·771)	(3·787)						
Kidneys‡	1	275	916	1469	1679	1560	—	(0·0588)	Overall	NS	***	NS
		(2·440)	(2·962)	(3·167)	(3·225)	(3·193)			Linear		***	•
	2	331	807	1178	1191	1102			Quadratic		***	NS
		(2·520)	(2·907)	(3·071)	(3·076)	(3·042)						
Pancreas‡	1+2	99	3639	2851	2080	2438	—	—	Overall	*	***	NS
		(1·995)	(3·561)	(3·455)	(3·318)	(3·387)	(0·0534)	—	Linear		***	NS
									Quadratic		***	NS
Spleen	1+2	29	44	44	40	33	3·0	—	Overall	***	**	NS
									Linear		NS	NS
									Quadratic		***	NS
Adrenals	1	1·5	2·0	2·1	2·9	2·4	—	0·25	Overall	*	NS	•
	2	1·8	1·9	2·2	1·6	1·7			Linear		NS	**
									Quadratic		NS	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Breed 1, Hisex white; breed 2, Hisex brown.
‡ Mean values are antilogs of the mean of the log transformations which are shown in parentheses.

Table 5. *Expt 1. Mean iron concentrations (µg/g dry matter) and total contents (µg) in some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets*

		Level of added Zn (g/kg)					SEM		Statistical significance of effect			
	Breed†	0	4	8	12	16	Diet	Breed × diet	Response	Breed	Diet	Breed × diet
Fe concentrations												
Liver‡	1	255	270	513	841	1042			Overall	*	***	NS
		(2·406)	(2·432)	(2·710)	(2·925)	(3·018)	—	(0·0468)	Linear		***	NS
	2	245	389	733	977	923			Quadratic		*	**
		(2·390)	(2·590)	(2·865)	(2·990)	(2·965)						
Kidneys	1+2	285	213	271	295	366	14·2	—	Overall	NS	***	NS
									Linear		***	NS
									Quadratic		***	NS
Pancreas	1	108	290	224	337	365			Overall	***	***	*
	2	97	197	235	246	196	—	27·9	Linear		***	*
									Quadratic		**	NS
Spleen	1+2	681	582	694	811	843	52·5	—	Overall	NS	**	NS
									Linear		**	NS
									Quadratic		NS	NS
Adrenals	1+2	153	132	117	117	147	9·4	—	Overall	NS	*	NS
									Linear		NS	NS
									Quadratic		**	NS
Total Fe contents												
Liver‡	1	2723	2483	3020	5321	5943			Overall	**	***	*
		(3·435)	(3·395)	(3·480)	(3·726)	(3·774)	—	(0·0461)	Linear		***	NS
	2	3062	3664	5012	5768	5200			Quadratic		NS	*
		(3·486)	(3·564)	(3·700)	(3·761)	(3·716)						
Kidneys	1+2	696	483	571	539	651	36·6	—	Overall	NS	***	NS
									Linear		NS	NS
									Quadratic		***	NS
Pancreas	1+2	108	176	143	161	146	12·1	—	Overall	NS	**	NS
									Linear		NS	NS
									Quadratic		*	NS
Adrenals	1+2	5·7	6·0	7·1	7·0	7·3	0·49	—	Overall	NS	NS	NS
									Linear		**	NS
									Quadratic		NS	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Breed 1, Hisex white; breed 2, Hisex brown.
‡ Mean values are antilogs of the mean of the log transformations which are shown in parentheses.

Table 6. *Expt 1. Mean copper concentrations (μg/g dry matter) and total contents (μg) in some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets*

		Level of added Zn (g/kg)					SEM		Statistical significance of effect			
	Breed†	0	4	8	12	16	Diet	Breed × diet	Response	Breed	Diet	Breed × diet
Cu concentrations												
Liver	1+2	12·0	9·3	12·8	12·8	14·5	0·62	—	Overall	*	***	NS
									Linear		***	NS
									Quadratic		*	NS
Kidneys	1+2	13·3	17·1	20·1	25·8	28·8	1·83	—	Overall	NS	***	NS
									Linear		***	NS
									Quadratic		NS	NS
Pancreas	1+2	3·7	6·5	6·6	6·5	7·6	0·43	—	Overall	NS	***	NS
									Linear		***	NS
									Quadratic		*	NS
Spleen	1+2	3·9	3·1	3·2	3·3	3·4	0·13	—	Overall	NS	**	NS
									Linear		NS	NS
									Quadratic		***	NS
Adrenals	1+2	3·5	1·8	2·2	2·8	3·9	0·30	—	Overall	NS	***	NS
									Linear		NS	NS
									Quadratic		***	NS
Total Cu contents												
Liver	1+2	136	87	82	79	81	4·0	—	Overall	***	***	NS
									Linear		***	NS
									Quadratic		***	NS
Kidneys	1+2	32	38	42	46	51	3·3	—	Overall	NS	**	NS
									Linear		***	NS
									Quadratic		NS	NS
Spleen	1+2	1·31	1·43	1·33	1·21	1·10	0·091	—	Overall	***	NS	NS
									Linear		*	NS
									Quadratic		NS	NS
Adrenals	1+2	0·14	0·09	0·13	0·17	0·19	0·018	—	Overall	NS	**	NS
									Linear		**	NS
									Quadratic		*	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Breed 1, Hisex white; breed 2, Hisex brown.

increased to 128% of the control value and total Fe content decreased by a maximum of 23%. The pancreas also showed a marked increase in Fe concentration and content.

Liver, pancreas, spleen and adrenals Cu concentrations (Table 6) exhibited a quadratic response and renal Cu concentrations showed a positive linear response. Total Cu content of the liver gave a decreasing quadratic response to dietary added ZnO whereas total content of the kidneys showed a positive linear response. Total pancreatic Cu (results not presented) was unaffected by treatment, the mean for the white and brown birds being 3·6 and 4·9 μg/g dry matter respectively.

Expt 2

The mortality rates of the birds offered diets containing 5 or 6 g added Zn/kg were 56 and 86% respectively. Consequently information concerning the mean performance and tissue mineral analysis of the birds on the highest level of dietary ZnO has been omitted from the results. Mild changes were observed in the gizzards of the white hens with small erosions apparent, particularly in the troughs of the rugae of the gizzard linings. More distinctive changes were observed in the gizzards of the brown strain including haemorrhages of the koilin layer and dilatation of the mucosal glands.

Zn intakes (Table 7) showed an increasing quadratic response. Mean food intakes, body-weights, egg production and food conversion efficiencies (Table 7) were significantly depressed by addition of ZnO. Mean food consumptions of the birds on the highest level of added ZnO were reduced by 64% compared with the controls and there was a negative linear relation between final body-weight and increasing dietary Zn concentration.

An almost complete pause in egg production was induced by 4 and 5 g added Zn/kg diet and significant linear reductions in egg number, total egg weight and food conversion efficiency were apparent in response to the rise in dietary ZnO.

The effects of dietary ZnO incorporation on the fresh weights of organs, expressed per unit body-weight, together with the liver lipid values are shown in Table 8. Significant overall dietary effects on liver, pancreas, gizzard, ovary and oviduct fresh weights/kg body-weight were observed. Liver lipid concentration and total content were significantly reduced by increasing added ZnO.

Results of the analyses of tissue Zn, Fe and Cu concentrations and total contents are shown in Tables 9–11 respectively. The liver and pancreatic Zn concentrations and total contents exhibited significant increasing quadratic responses to dietary ZnO levels, while kidney and spleen concentrations and kidney total Zn showed increasing linear responses.

The liver Fe concentrations and contents increased by up to 200% at the highest level of Zn addition for the white birds. In contrast, there was a decrease at the intermediate levels of addition of about 50% for both strains. Kidney Fe concentrations and contents were depressed by ZnO treatments except at the 1 g/kg level of addition.

The Cu concentrations and total contents of the liver were both significantly reduced by dietary treatment. The Cu concentrations of the kidneys and pancreas showed quadratic relations to dietary treatment. Negative linear responses were observed for the Cu concentration and total content of the spleen.

DISCUSSION

The results confirm previous evidence (Creger & Scott, 1977; Shippee *et al.* 1979) that high dietary levels of ZnO can be used to induce a pause in lay by the mature, female domestic fowl.

Although Shippee *et al.* (1979) advocated the use of 10 g Zn as ZnO/kg diet, it is evident from Expt 1 that the addition of between 4 and 8 g Zn as ZnO/kg diet is sufficient to ensure

Table 7. *Expt 2. Mean food and zinc intakes, body-weights, egg production values and food conversion efficiency of mature, female domestic fowl given control and zinc oxide-supplemented diets for five 28 d periods*

		Level of added Zn (g/kg)						SEM		Statistical significance of effect			
	Breed†	0	1	2	3	4	5	Diet	Breed × diet	Response	Breed	Diet	Breed × diet
Food intake (kg)	1+2	3·45	3·48	3·00	2·19	1·88	1·25	0·088	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		**	NS
Zn intake‡ (mg)	1+2	239 (2·379)	3365 (3·527)	5728 (3·758)	5957 (3·775)	6622 (3·821)	5888 (3·770)	(0·0159)	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		***	NS
Initial body-wt (kg)	1+2	1·87	1·87	1·88	1·88	1·89	1·88	0·013	—	Overall	***	NS	NS
										Linear		NS	NS
										Quadratic		NS	NS
Final body-wt (kg)	1	1·47	1·54	1·40	1·31	1·10	1·07	—	0·072	Overall	***	***	***
	2	2·20	2·18	1·94	1·70	1·48	1·42			Linear		***	***
										Quadratic		NS	NS
Egg no.	1+2	21·7	21·6	15·9	6·1	2·3	1·7	0·92	—	Overall	*	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Total egg weight (kg)	1+2	1·31	1·32	0·97	0·35	0·13	0·10	0·058	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
FCE	1+2	0·390	0·380	0·324	0·172	0·073	0·090	0·0217	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS

NS, not significant; FCE, food conversion efficiency (kg eggs produced/kg food intake).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Breed 1, Hisex white; breed 2, Hisex brown.
‡ Mean values are antilogs of the mean of log transformations which are shown in parentheses.

Table 8. *Expt 2. Mean fresh weights of some tissues expressed as g/kg body-weight, together with the liver lipid concentration (g/kg dry matter and total liver lipid content (g) of mature, female domestic fowl given control and zinc oxide-supplemented diets for five 28 d periods*

	Breed†	Level of added Zn (g/kg) 0	1	2	3	4	5	SEM Diet	SEM Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Tissue weights													
Liver	1+2	18·8	18·5	21·1	17·7	15·5	16·8	0·99	—	Overall	***	**	NS
										Linear		*	NS
										Quadratic		NS	NS
Pancreas	1+2	1·74	1·56	1·50	1·08	1·27	1·35	0·128	—	Overall	***	*	NS
										Linear		**	NS
										Quadratic		*	NS
Spleen	1	0·74	0·55	0·68	0·79	0·92	1·00	—	0·102	Overall	NS	NS	NS
	2	1·01	0·77	0·84	0·83	0·78	0·82			Linear		NS	**
										Quadratic		NS	NS
Gizzard	1+2	14·0	12·7	17·2	17·9	25·5	26·4	1·11	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		*	NS
Ovary	1+2	24·5	24·9	18·5	14·4	1·3	1·3	2·06	—	Overall	***	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Oviduct	1	37·0	35·3	36·4	23·5	2·3	2·6	—	2·32	Overall	***	***	**
	2	25·3	24·9	20·9	8·3	1·4	1·4			Linear		***	**
										Quadratic		**	*
Liver lipid Concentration	1+2	175	182	192	146	116	116	13·4	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Total content	1+2	1·64	1·78	1·86	1·08	0·65	0·65	0·200	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Breed 1, Hisex white; breed 2, Hisex brown.

Table 9. *Expt 2. Mean zinc concentrations (μg/g dry matter) and total contents (μg) in some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets for five 28 d periods*

	Breed†	Level of added Zn (g/kg) 0	1	2	3	4	5	SEM Diet	SEM Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Zn concentrations													
Liver‡	1+2	143	333	1033	1225	1542	1469		—	Overall	NS	***	NS
		(2·154)	(2·522)	(3·014)	(3·088)	(3·188)	(3·167)	(0·0332)	—	Linear		***	NS
										Quadratic		***	NS
Kidneys	1+2	136	143	252	409	629	624	37·9	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Pancreas‡	1	108	339	2404	3443	3715	3289			Overall	NS	***	NS
		(2·034)	(2·530)	(3·381)	(3·537)	(3·570)	(3·517)	—	(0·0704)	Linear		***	*
	2	93	340	2742	3451	3281	3784			Quadratic		***	NS
		(1·970)	(2·532)	(3·438)	(3·538)	(3·516)	(3·578)						
Spleen	1+2	80	83	87	93	120	113	2·7	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Total Zn contents													
Liver‡	1+2	1285	3228	9354	8954	8492	7980		—	Overall	NS	***	NS
		(3·109)	(3·509)	(3·971)	(3·952)	(3·929)	(3·902)	(0·0468)	—	Linear		***	NS
										Quadratic		***	NS
Kidneys‡	1+2	360	365	577	769	1202	935		—	Overall	NS	***	NS
		(2·556)	(2·562)	(2·761)	(2·886)	(3·080)	(2·971)	(0·0422)	—	Linear		***	NS
										Quadratic		NS	NS
Pancreas‡	1+2	101	292	1656	1574	1538	1517		—	Overall	NS	***	NS
		(2·003)	(2·465)	(3·219)	(3·197)	(3·187)	(3·181)	(0·0643)	—	Linear		***	NS
										Quadratic		***	NS
Spleen	1	22	17	20	26	29	30			Overall	***	NS	NS
	2	42	34	32	30	34	32	—	4·5	Linear		NS	*
										Quadratic		NS	NS

NS, not significant.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† Breed 1, Hisex white; breed 2, Hisex brown.
‡ Mean values are antilogs of the mean of log transformations which are shown in parentheses.

Table 10. *Expt 2. Mean iron concentrations (µg/g dry matter) and total contents (µg) in some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets for five 28 d periods*

		Level of added Zn (g/kg)						SEM		Statistical significance of effect			
	Breed†	0	1	2	3	4	5	Diet	Breed × diet	Response	Breed	Diet	Breed × diet
Fe concentrations													
Liver	1	375	364	197	211	440	784	—	32·3	Overall	NS	***	***
	2	427	428	252	294	533	441			Linear		***	***
										Quadratic		***	***
Kidneys	1	338	356	219	199	217	227	—	18·5	Overall	***	***	*
	2	231	254	196	199	174	209			Linear		***	**
										Quadratic		**	NS
										Overall	*	***	NS
Pancreas	1+2	93	160	174	171	160	199	11·0	—	Linear		***	NS
										Quadratic		*	NS
										Overall	***	***	NS
Spleen	1+2	801	841	686	640	621	631	27·8	—	Linear		***	NS
										Quadratic		NS	NS
Total Fe contents										Overall	NS	***	***
Liver	1	3127	3429	2096	1711	2299	4484	356·2	—	Linear		*	***
	2	4189	4355	2137	1974	3049	2339			Quadratic		***	NS
Kidneys	1	904	879	509	366	410	380	—	63·4	Overall	*	***	NS
	2	622	690	465	425	344	314			Linear		***	*
										Quadratic		*	NS
										Overall	NS	*	NS
Pancreas	1+2	94	143	119	86	74	89	15·7	—	Linear		*	NS
										Quadratic		NS	NS
Spleen	1	239	187	163	197	175	188	—	39·9	Overall	*	**	NS
	2	381	329	247	209	154	164			Linear		***	**
										Quadratic		NS	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Breed 1, Hisex white; breed 2, Hisex brown.

Table 11. *Expt 2. Mean copper concentrations (μg/g dry matter) and total contents (μg) in some tissues of mature, female domestic fowl given control and zinc oxide-supplemented diets for five 28 d periods*

	Breed†	Level of added Zn (g/kg) 0	1	2	3	4	5	SEM Diet	SEM Breed × diet	Statistical significance of effect: Response	Breed	Diet	Breed × diet
Cu concentrations													
Liver	1	15·7	16·9	7·6	7·2	8·1	7·7	—	0·85	Overall	NS	***	NS
	2	13·5	14·6	7·8	8·9	7·7	8·8			Linear		***	*
										Quadratic		***	NS
Kidneys	1+2	11·8	11·8	14·7	14·4	18·8	14·4	0·77	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		*	NS
Pancreas	1+2	4·4	5·2	6·8	6·0	3·9	4·5	0·37	—	Overall	NS	***	NS
										Linear		NS	NS
										Quadratic		***	NS
Spleen	1+2	4·3	4·8	3·9	3·3	3·5	3·2	0·19	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Total Cu contents													
Liver	1+2	132	153	72	60	44	44	6·8	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		*	NS
Kidneys	1+2	32	30	34	28	37	23	2·4	—	Overall	NS	**	NS
										Linear		NS	NS
										Quadratic		NS	NS
Pancreas	1+2	4·5	4·5	4·5	3·1	1·8	2·1	0·39	—	Overall	NS	***	NS
										Linear		***	NS
										Quadratic		NS	NS
Spleen	1	1·18	1·03	0·91	0·92	0·82	0·84	—	0·174	Overall	***	***	NS
	2	2·24	1·82	1·36	1·12	1·07	0·92			Linear		***	**
										Quadratic		NS	NS

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Breed 1, Hisex white; breed 2, Hisex brown.

a rapid cessation in egg production and act effectively as a technique for the force-resting of laying hens.

The relatively high degree of tolerance of adult hens to excessive levels of dietary Zn compounds over an extended period of time was clearly demonstrated by Expt 2 which indicated that food consumption, body-weight and egg production over a 5-month period were not significantly affected by the dietary incorporation of up to 1 g Zn as ZnO/kg. These observations corroborated those of Hermayer *et al.* (1977) who reported similar results.

The severe depression of food intake at dietary levels of ZnO addition in excess of 1 g Zn/kg diet presumably contributed to the corresponding reductions in body-weight and egg numbers, since the intakes of major and minor nutrients were well below the specific requirements recommended by the Agricultural Research Council (1975) for maintenance and adequate production. However, it is not clear whether the resultant loss in appetite associated with high dietary levels of ZnO may be attributed to toxic effects of the compound or to a reduction in palatability. The domestic fowl has been reported to have relatively few taste buds (Kare & Rogers, 1976) and, in view of the organoleptic nature of ZnO, it would seem that factors other than just a decrease in palatability have, at least partially, induced inappetance. Dewar *et al.* (1983) have identified gizzard and pancreatic lesions occurring in hens offered 10 g Zn as ZnO/kg diet for 4 d, and it is possible that these toxic effects may be injurious to the general health and consequently to the appetite of the bird.

In the short-term experiment (Expt 1), further reductions in body-weight were not substantially induced by dietary addition of ZnO at levels providing more than 8 g Zn/kg diet. Examination of the fresh weights of the liver, pancreas and reproductive organs per unit body-weight also revealed that they were not significantly further depressed by higher dietary levels of added ZnO despite associated reductions in food intake. However, it should also be noted that Zn intake per unit body-weight was maximum for the brown strain at 4 g added Zn/kg diet while for the white strain the maximum was above 8 g added Zn/kg. It appears that offering laying hens a diet containing approximately 8 g Zn/kg for at least 7 d is sufficient to cause a complete regression in the ovary and oviduct tissues. In the fowl the liver is the principal site of fatty acid synthesis (Leveille, 1969). Thus, a considerable proportion of the decrease in liver fresh weight may be attributed to the depression of oestrogen production and the consequent inhibition of oestrogen-induced lipidaemia (Griminger, 1976). Furthermore, the decrease in liver weight was similar in magnitude to the increase in hepatic weight observed when immature pullets came into lay (Pearce, 1971).

The marked increase in gizzard weight in response to dietary ZnO addition is rather similar to the effects observed on supplementing diets with high levels of copper sulphate (Fisher *et al.* 1973; Stevenson & Jackson, 1981).

Brake *et al.* (1977) suggested that increases in adrenal gland and spleen weight of force-moulted birds were indicative of physiological stress, at least during the early phase of starvation, while Eltohamy *et al.* (1980) identified hypertrophy of the adrenal cortex in cockerels offered up to 4 g Zn/kg diet and hypothesized that excessive dietary Zn indirectly affected the release of adrenocorticotrophic hormone from the amphophils of the cephalic lobe. This suggests that the increased weights of the adrenals and spleen per unit body-weight may possibly have been induced by stress effects, thus reducing food consumption.

The increase in hepatic and renal Zn concentration with increasing dietary level of ZnO addition supports the findings of Johnson *et al.* (1962) and Kincaid *et al.* (1976*b*) who reported elevated Zn concentrations in both the liver and kidneys of broiler chicks given diets incorporating ZnO at levels of 2 and 2·4 g Zn/kg diet respectively.

The highest concentration of Zn was found in the pancreas, increasing by 500-fold in hens offered 4 g Zn as ZnO/kg diet. This substantiates the observation by Oh *et al.* (1979) that Zn accumulated to the largest extent in the metallothionein of pancreatic tissue in broiler chicks offered excess dietary Zn acetate. Marked increases in pancreatic Zn have also been reported in cockerels (Eltohamy *et al.* 1980), sheep (Ott *et al.* 1966*a*) and calves (Kincaid *et al.* 1976*a*) given high levels of Zn compounds.

It is apparent from the present findings that the accumulation of tissue Zn in the fowl exhibits a threshold level of tolerance to the concentration of dietary ZnO. The results of Expt 2 indicate that the threshold for mature hens given ZnO-supplemented diets is at a level of about 1 g Zn/kg diet. Although at this level of inclusion the Zn concentrations of both liver and pancreas were observed to increase more than twofold compared with the controls, the effect of 2 g added Zn as ZnO/kg diet was to increase their Zn concentrations by over seven- and twenty-five-fold respectively. Thresholds of dietary Zn compound concentrations above which tissue Zn accumulation increases markedly have also been identified for various species (Ott *et al.* 1966*b*; Kincaid *et al.* 1976*b*; Hamilton *et al.* 1979). It is important in making comparisons to realize that the threshold levels are largely dependent on the physical nature and solubility of the Zn compound.

The quadratic response of liver Fe content to increasing dietary levels of supplemental ZnO observed in Expt 2 was unexpected. Previous investigations with other species such as rats (Cox & Harris, 1960; Magee & Matrone, 1960), swine (Cox & Hale, 1962) and Japanese quail (*Coturnix coturnix japonica*) (Hamiliton *et al.* 1979) have shown that excessive levels of dietary Zn compounds cause a marked reduction in hepatic Fe concentration and content. However, the anomalous results obtained for the domestic fowl may be partly explained in terms of the corresponding effects of the different dietary treatments on egg production. The depletion of hepatic Fe content observed for the lower levels of ZnO inclusion may have been due to the joint effect of a diminished tissue Fe uptake concomitant with a drain of liver Fe stores associated with egg production. Egg-yolk formation has been shown by Halkett *et al.* (1958) to draw on the plasma Fe pool in laying hens and, since it has been estimated that a 50 g egg contains approximately 2·25 mg Fe (Romanoff & Romanoff, 1949), it would seem likely that egg production draws heavily on their Fe reserves. The observed increases in liver Fe content for the groups offered the diets with the two highest levels of ZnO supplementation may therefore reflect the corresponding cessation in egg production induced by these treatments. The resultant effect on the hepatic Fe content may have been reinforced by the associated depletion of liver Cu stores since there is evidence that Fe mobilization is inhibited at low Cu levels (Butler, 1971).

The slight increase observed in liver Cu concentration with the high dietary ZnO treatments of Expt 1 was evidently a reflection of the diminished liver weight since added dietary ZnO caused a significant depression in total liver Cu content. The severe reduction in hepatic Cu levels was even more apparent in Expt 2. The distinct increase in kidney total Cu content and concentration may reflect an increase in renal excretion of endogenous Cu associated with the observed antagonistic effect of dietary ZnO on liver Cu storage. The fall in the spleen and adrenal Cu concentrations at the 4 g Zn/kg level of supplementation may be attributed to the competitive effect of Zn and Cu but there is no obvious explanation for the rise in Cu concentration of the adrenals after the initial fall at the lowest level of supplementation although this must be a function of treatment.

The effects of excessive dietary ZnO intake on the storage and utilization of Cu in the mature fowl have not been investigated by other workers, although the antagonistic effect of dietary ZnO on tissue Cu accumulation has been extensively reported for other species including rats (Cox & Harris, 1960), broiler chicks (Johnson *et al.* 1962), sheep (Ott *et al.* 1966*b*) and turkey poults (Vohra & Heil, 1969). Furthermore, an inverse correlation between hepatic Fe and Cu levels has been identified by Ritchie *et al.* (1963) in growing pigs as well as by Ott *et al.* (1966*b*) in lambs. The results of the present work suggest that an inverse relation between the liver total Fe and Cu contents also exists in adult domestic fowl offered high levels of dietary ZnO.

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