The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels of the mature, laying, domestic fowl

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1. A cereal-based diet containing 16 mg copper/kg was fed *ad lib.* to a group of laying hens for 35 d. Five other groups were given this control diet to which was added 120, 240, 480, 960 and 1920 mg Cu/kg (as copper sulphate).

2. Records were kept of daily food intake, water intake and egg production.

3. After 35 d the hens were slaughtered and blood haemoglobin, packed cell volume, Cu and aspartate aminotransferase (*EC* 2.6.1.1) levels assayed. Liver, oviduct, kidney and breast muscle Cu and iron concentrations were measured.

4. Food and water intakes were depressed by the two highest levels of dietary Cu and water intake was increased by the diet with 240 mg added Cu/kg. Both food and water intake showed a quadratic relationship with the level of added dietary Cu.

5. Body-weight loss was increased by the addition of Cu and showed a significant linear relationship with the concentration of added Cu in the diet. Liver and oviduct weights were depressed at the two highest levels of Cu addition.

6. Liver and oviduct Cu and Fe concentrations were significantly increased by high dietary Cu and mean total liver and kidney Cu and Fe showed an increase although for the liver this was not statistically significant.

Copper has been shown to be highly toxic for certain animals including sheep and cattle (Shand & Lewis, 1957; Todd & Thompson, 1963), while the pig can tolerate quite high levels (500 mg/kg diet) of Cu at least for short periods (Allcroft, Burns & Lewis, 1961; Allen & Harding, 1962). There is little information available regarding the effect of Cu in fowl and the limited number of experiments to date on the domestic fowl have mainly been concerned with the effect of Cu in the chick.

In general up to about 250 mg Cu/kg diet tends to promote the growth of chicks (Mayo, Hauge, Parker, Andrews & Carrick, 1956; Smith, 1969; Jenkins, Morris & Valamotis, 1970), while above this a growth depression occurs and at very high levels considerable mortality may occur (Mayo *et al.* 1956; Mehring, Brumbaugh, Sutherland & Titus, 1960). King (1972) found that the weight of the small intestine of chicks was decreased and the weight of the caecums increased, on a body-weight basis, when the dietary Cu was increased.

Goldberg, Williams, Jones, Yanagita, Cartwright & Wintrobe (1956) gave 100 mg Cu daily as acetate (equivalent to 1000 mg Cu/kg diet) to adult White Leghorn hens. About 30 % of the hens developed anaemia. The control birds had plasma Cu levels of 330 μg/l and the anaemic birds levels of 150–7620 μg/l. Rangachar & Hedge (1973) gave 10 mg Cu/d as sulphate, to 14-week-old White Leghorn pullets, and found, after 30 d, higher packed cell volume (PCV) and haemoglobin levels, but no effect on growth rate.

Giving diets high in Cu to fowl results in increased liver Cu concentrations (Goldberg *et al.* 1956; Mehring *et al.* 1960; Beck, 1961; Norvell, Calvert, Thomas & Goatcher, 1974) but there is a paucity of information on the effect of high dietary Cu on other cations, including iron.

Table 1. *Composition (g/kg) of the control diet (Treatment 1) given to laying hens*

Ground wheat	88·8
Ground maize	600·0
Peruvian fish meal	50·0
Soya-bean meal	150·0
Grass meal	25·0
Limestone flour	68·8
Dicalcium phosphate	9·8
Sodium chloride	4·0
Vitamin–mineral supplement*	3·6
Analysis (/kg)	
Dry matter (g)	889
Determined metabolizable energy (MJ)	11·4
Determined crude protein† (g)	165
Determined calcium (g)	38·9
Determined phosphorus (g)	6·7
Determined copper (mg)	16

* The vitamin-mineral supplement 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; 14·4 mg nicotinic acid; 5·8 mg pantothenic acid; 0·4 mg pteroylmonoglutamic acid; 72 mg choline chloride; 14·4 mg Fe; 2·2 mg Co; 72 mg Mn; 3·6 mg I; 72 mg Zn and 5·8 mg Cu.

† Nitrogen × 6·25.

In view of the lack of information on the effects of Cu on the adult domestic fowl it was decided to investigate the effect of high levels of dietary Cu on mature, laying hens.

EXPERIMENTAL

Twenty-four 1-year-old Shaver 288 laying hens were placed in individual galvanized iron metabolism cages and maintained on a lighting programme of 17 h light and 7 h darkness. The hens were randomized into six treatment groups each comprising four hens, and the birds of each treatment group were given one of the six experimental diets. The diets, fed *ad lib.* were the control diet (Treatment 1), the composition and analysis of which are presented in Table 1, and the control diet to which was added 120, 240, 480, 960 and 1920 mg Cu/kg as copper sulphate ($CuSO_4.5H_2O$) (Treatments 2–6 respectively). The hydrated copper sulphate was ground so that 30 % was less than 90 μm, 52 % less than 150 μm and 68 % less than 200 μm in size. Glass-distilled water was available at all times and had negligible Cu and Fe concentrations.

During the experiment daily records were kept of egg production, food consumption and water intake. Body-weight was recorded initially and at the end of the experiment.

The hens were all laying at the start of the experiment, which lasted for 35 d. At the end of this time the birds were slaughtered by decapitation. Blood haemoglobin, PCV, blood Cu and serum aspartate aminotransferase (AAT) (*EC* 2.6.1.1) were measured. The liver, oviduct, kidneys and a portion of breast muscle were removed, weighed and analysed for dry matter (DM), Cu and Fe content.

The total Cu in whole blood was determined by wet-ashing the blood with concentrated nitric and perchloric acids followed by atomic absorption spectroscopy (Thompson & Blanchflower, 1971). The Cu and Fe of the other tissues were determined by atomic absorption spectroscopy subsequent to dry ashing and solution in dilute HCl. Serum AAT was assayed by the method of Bergmeyer & Bernt (1965), and the results expressed in international units (i.u.) when one i.u. refers to the oxidation of 1 μmol of NADPH/min at 37 °.

The results were subjected to analysis of variance, log transformations being carried out

Table 2. *Mean food, water and supplementary copper intake, body-weight and egg production of laying hens given control and Cu-supplemented diets for 35 d*

	Treatment no.*						SE of Mean
	1	2	3	4	5	6	
Dietary Cu concentration (mg/kg)	16	136	256	496	976	1936	
Total supplementary Cu intake (g)	0	0·52	1·00	1·69	2·76	3·03	
Body-weight (kg)							
Initial	1·68	1·80	1·64	1·80	1·69	1·76	0·073
Final	1·65	1·82	1·69	1·66	1·55	1·38	0·077
Mean body-weight change (g)	−23	24	45	−141	−138	−384	55·91
Total food intake (kg)	3·92	4·32	4·16	3·53	2·87	1·58	0·157
Total water intake (l)	7·58	8·89	10·01	8·11	5·31	3·17	0·796
Mean no. of eggs	30·7	30·7	32·2	29·0	15·7	8·5	
T_{50} (time to lay 50 % of eggs (d))	13·8	13·1	12·9	13·0	7·9	4·4	
$\text{Log}_{10}\ T_{50}$	1·1410	1·1184	1·1122	1·1144	0·8978	0·6465	0·0384

*See p. 94 for explanation of treatments.

for those variables which were considered to exhibit variance heterogeneity. For egg production a form of probit analysis for each bird gave an estimate of the average time taken in each treatment to produce 50 % of the total eggs laid during the experimental period. This measure was used in preference to the time at which laying ceased since this latter tended to be very variable due to occasional eggs being layed after the end of the main egg-laying period.

RESULTS

No birds died in the experiment. The mean initial and final body-weights, body-weight gain, food and water intake, Cu intake and egg production are given in Table 2. The control group (Treatment 1) and the groups with the two lowest levels of copper sulphate addition (Treatments 2 and 3) showed little body-weight change. However, higher levels of copper sulphate addition caused a marked body-weight loss and at the highest level of addition the birds lost, on average, 22 % of their initial body-weight. Body-weight gain showed a significant linear relationship ($P < 0{\cdot}001$) with dietary concentration of added copper.

The daily food and water intakes were both very markedly depressed for the high-copper sulphate groups (Treatments 4, 5 and 6) from the start of the experiment. The food intake over the experimental period for Treatments 5 and 6 was statistically significantly lower ($P < 0{\cdot}001$) than for the other four treatment groups. Total water intake was significantly increased in Treatment 3 ($P < 0{\cdot}05$) and significantly decreased in Treatment 6 ($P < 0{\cdot}01$) when compared with the controls. This was reflected in the nature of the excreta, which tended to be quite wet for Treatment 3 and dry and green for Treatment 6. When analysed for trends total feed intake (kg) and total water intake (l) both showed a quadratic relationship with the level of added dietary copper (mg/kg) ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively). Mean egg production was affected at the two highest levels of Cu addition. At 14 d egg production for Treatment 5 became infrequent and for Treatment 6 it ceased altogether.

Table 3. *Liver and oviduct fresh and dry weights and dry matter* (DM) *content and blood haemoglobin, packed cell volume* (*PCV*) *and serum aspartate aminotransferase* (*AAT*) (EC 2.6.1.1) *of laying hens given control and copper-supplemented diets for* 35 *d*

	Treatment no.*						SE of Mean
	1	2	3	4	5	6	
Liver wt (g)							
Fresh	32·8	38·5	39·9	29·9	22·5	17·3	3·51
Dry	12·1	14·9	17·7	10·7	6·9	5·0	3·23
Liver DM (mg/g)	361	370	415	347	305	288	44·1
Oviduct† wt (g)							
Fresh	54·7 (1·738)	49·5 (1·695)	51·6 (1·713)	55·3 (1·743)	35·1 (1·545)	4·3 (0·635)	(0·0819)
Dry	13·8 (1·140)	14·1 (1·150)	12·9 (1·112)	13·9 (1·142)	7·9 (0·900)	1·1 (0·038)	(0·914)
Oviduct DM (mg/g)	234	297	252	252	225	253	23·8
Kidney fresh wt (g)	10·1	10·8	9·9	7·8	8·1	7·1	0·938
Blood haemoglobin (g/l)	72·8	70·8	72·8	72·3	85·5	76·5	5·34
PCV (%)	22·7	23·0	23·2	24·5	27·5	24·7	1·642
Mean corpuscular haemoglobin concentration (g/dl)	31·7	30·7	31·0	29·2	31·0	31·2	1·074
Serum AAT (i.u./l)	227	215	336	263	320	357	44·0
Blood Cu (μg/l)	300	390	840	750	370	440	258

* See p. 94 for explanation of treatments.
† Oviduct weight analysis of variance carried out using log transformations. The mean values for oviduct weight presented are the anti-logs of the means of the log transformation.

The liver, oviduct and kidney weights and liver and oviduct DM contents are presented in Table 3 together with the whole blood analysis and serum AAT values. PCV, mean corpuscular haemoglobin content and blood Cu were not affected by treatment and although the haemoglobin and serum AAT values appeared to be increased by dietary Cu these effects were not statistically significant.

The liver fresh weight showed a marked depression at the two highest levels of copper sulphate addition ($P < 0{\cdot}01$). A decrease in mean liver DM at the two highest levels of Cu addition occurred but statistical significance was not attained.

Oviduct weight was depressed ($P < 0{\cdot}001$) as a result of the copper sulphate addition, the fresh and dry oviduct weight for Treatment 6 only being some 8 % of that for the control ($P < 0{\cdot}001$). The mean value for Treatment 5 would have been further depressed but for the fact that one bird in this group had the heaviest oviduct of the experiment, the four oviduct weights in Treatment 5 being 38·8, 82·3, 42·7 and 11.2 g.

Kidney weight tended to be lower as a result of dietary Cu supplementation but statistical significance was not attained.

The Cu and Fe analysis of liver, oviduct, kidney and breast tissue are shown in Table 4. The liver Cu and Fe levels were increased by dietary copper sulphate level ($P < 0{\cdot}01$). For the two highest copper sulphate treatments the mean liver Cu concentrations showed significant increases over most of the other treatments although the individual values varied considerably for all treatments. Liver Fe concentration was also high in Treatment 6, being statistically greater than for Treatments 2, 3, 4 ($P < 0{\cdot}001$) and 5 ($P < 0{\cdot}01$) as well as the

Table 4. *Copper and iron concentrations and contents in liver, oviduct, kidney and breast muscle of laying hens given control and Cu-supplemented diets for* 35 *d*

	Treatment no.*						SE of Mean
	1	2	3	4	5	6	
Liver DM							
Cu concentration† (ug/g)	17 (1·225)	17 (1·237)	5 (0·680)	36 (1·562)	291 (2·464)	558 (2·747)	(0·3729)
Total Cu content† (mg)	0·19 (−0·714)	0·23 (−0·632)	0·07 (−1·136)	0·37 (−0·433)	1·99 (0·300)	2·77 (0·444)	(0·3762)
Fe concentration (μg/g)	444	232	239	299	500	1573	211·4
Total Fe content (mg)	4·25	3·14	3·28	2·95	3·48	7·92	0·706
Oviduct DM							
Cu concentration (μg)	4·2	7·4	3·4	10·0	8·9	22·4	3·355
Total Cu content (μg/g)	44	109	41	129	88	21	36·8
Fe concentration (μg/g)	34	24	33	41	51	105	8·73
Total Fe content (μg)	480	336	421	540	395	115	69·1
Kidney (fresh)							
Cu concentration† (μg/g)	3·9 (0·593)	2·6 (0·408)	4·4 (0·642)	4·3 (1·633)	12·8 (1·107)	20·4 (1·309)	(0·7822)
Total Cu content† (μg)	39·3 (1·594)	27·0 (1·431)	43·1 (1·634)	33·0 (1·518)	101·6 (2·007)	142·6 (2·154)	(0·2424)
Fe concentration (μg/g)	85	59	94	76	90	350	87·6
Total Fe content (μg)	830	632	925	600	730	2237	235·4
Breast muscle (fresh)							
Cu concentration† (μg/g)	0·17 (−0·771)	0·61 (−0·215)	0·43 (−0·357)	1·56 (0·194)	0·40 (−0·402)	0·59 (−0·230)	(0·4104)
Fe concentration (μg/g)	3·9	2·5	3·5	3·9	3·8	3·8	0·48

* See p. 94 for explanation of treatments.

† Analysis of variance carried out using log transformations. The mean values presented are the anti-logs of the mean of the log transformations. The figures in parentheses are the means of the log data.

control ($P < 0·01$). The control Cu and Fe concentrations did not differ significantly from any treatment values other than those of Treatments 5 and 6.

The total Cu content of the liver was shown not to be significantly affected by treatment although the means strongly suggest an increase in total liver Cu content with increasing dietary level. The total Fe content was significantly affected ($P < 0·001$). With respect to total Cu and Fe content, the increases in mean liver Cu and Fe concentration appear to more than compensate for the decrease in liver size, so that the mean total liver Cu content for Treatments 5 and 6 were considerably greater than for the other treatments. The mean total liver Fe content of Treatment 6 was statistically greater than that for the control ($P < 0·01$) and the other four treatments ($P < 0·001$).

Oviduct Cu concentration increased with copper sulphate treatment ($P < 0·01$), as did Fe concentration. Total oviduct Cu content was not affected by treatment, while the total Fe content tended to be depressed ($P < 0·01$).

Mean kidney Cu and Fe concentration and Cu content increased with dietary Cu level but

when analysis of variance was applied, statistical significance was not attained. Total kidney Fe content was significantly increased in Treatment 6 (P 0·001).

The concentrations of Cu and Fe in the breast tissue were not significantly affected by treatment.

DISCUSSION

Although the immature domestic fowl is known to be much more tolerant of high dietary level of Cu than ruminant species (Mayo *et el.* 1956; Smith, 1969) nevertheless, in the present experiment, the lack of mortality at the very high levels of dietary copper sulphate was unexpected in view of the reported toxicity for growing chicks (Mayo *et al.* 1956; Mehring *et al.* 1960).

The results for body-weight are in fair agreement with results obtained for hens (Goldberg *et al.* 1956), chicks (Mehring *et al.* 1960; Smith, 1969; King, 1972) and ducklings (King, 1975), in that low levels of Cu tended to promote growth while high levels depressed growth. However, the position in the mature hen is rather different to that in the young, growing chick. In the former, at the high levels of Cu addition, about 20 % of the weight loss could be attributed to liver weight loss and the marked oviduct regression. The body-weight loss in Treatments 4, 5 and 6 can be attributed mainly to the decrease in food intake which became apparent, especially for Treatments 5 and 6, within 2 d of introducing the hens to the copper sulphate-containing diets. Using accepted values for the maintenance and production metabolizable energy requirements of the laying hen (Waring & Brown, 1967) it can be shown that the Treatment 6 birds were consuming well below their maintenance requirement, while the Treatment 4 and 5 birds were only consuming enough energy to allow for production well below their potential.

The lack of any statistically significant effect of Cu intake on blood haemoglobin, PCV, mean corpuscular haemoglobin content and blood Cu (Table 3) does not conform with the results of Goldberg *et al.* (1956), Rangachar & Hedge (1973) or the recent observation of Kuznetsov & Volkov (1974), who found that 145 mg Cu/kg diet, as the sulphate, caused a 30 % increase in plasma Cu concentration.

Serum AAT is known to be elevated in cases of human liver dysfunction (Norberg, 1961) and in sheep highly elevated levels of alanine aminotransferase (*EC* 2.6.1.2) and AAT have been found about the time of haemolytic crisis associated with Cu poisoning (Todd & Thompson, 1963).

Although the blood analysis did not show statistically significant effects there is reason to believe that with a larger number of animals per treatment the trends in haemoglobin and serum AAT, which appear to be due to treatment, would have proved significant. Also, a longer experimental period would probably have shown clinical evidence of toxicity, at least in Treatment 6. Although haemoglobin was not significantly affected for Treatments 5 or 6, there may have been a greater turnover of haemoglobin in these treatments, the Fe thus made available being stored in the liver and kidney. In addition, liver and kidney storage may be enhanced as a result of the lack of demand for Fe for egg production in Treatments 5 and 6.

The effect of high levels of dietary Cu in increasing liver Cu concentration and total content in the present experiment agrees with results obtained for other fowl. Mehring *et al.* (1960) found mean liver Cu concentration to increase from 14 μg/g in a control (26 mg Cu/kg diet) to 820 μg/g in a group receiving 1176 mg Cu/kg diet. Beck (1961) fed cockerels and ducks on diets containing up to 27 mg Cu/kg as copper sulphate for 12 weeks. For immature cockerels the mean initial fat-free liver Cu concentration was 13·5 μg/g and the maximum after 12 weeks was 16·3 μg/g. This effect was much greater in the immature duck, the initial and final fat-free liver copper concentration being 39 and 183 μg/g respectively.

Since the present experiment was carried out Norvell *et al.* (1974) found for broiler chickens that liver Cu was directly related to Cu supplementation and Russian workers (Kuznetsov & Volkov, 1974) have published results on the feeding of up to 145 mg added Cu, as copper sulphate/kg diet to Leghorn hens for up to 270 d. The 145 mg Cu/kg supplement increased liver Cu by about 50 %. The effect on the fowl would appear to be rather similar to that in rats, where liver Cu storage is not affected until high dietary Cu concentrations (200 mg/kg) are reached (Milne & Weswig, 1968).

The increase in liver Fe with high dietary Cu may seem at variance with the results of other workers who found a highly significant inverse correlation between hepatic Fe and Cu concentration in the rat (Sourkes, Lloyd & Birnbaum, 1968). However, this relationship was found at low levels of Cu and Fe in the diet whereas the present work is concerned with effects at high levels of Cu supplementation. Kuznetsov & Volkov (1974) found liver Fe to be increased by added levels of Cu of 45 and 145 mg/kg diet, although in time the effect was lost.

The Fe concentration in the oviduct showed a marked increase at the highest level of Cu addition but because of the marked oviduct regression the total content was much lower for Treatment 5 and 6 than for the other treatments.

Although the accumulation of Cu or Fe in the kidney as a result of treatment both failed to attain statistical significance a study of the individual values leaves little doubt that the high mean values for Treatment 6 were real effects. The lack of any treatment effect on the breast Cu or Fe content is in agreement with results of Kuznetsov & Volkov (1974), although Norvell *et al.* (1974) have reported an increase in breast Cu in the female broiler chicken given up to 720 mg Cu/kg diet.

In the domestic fowl the liver is the principal site of fatty acid synthesis (Goodridge, 1968; O'Hea & Leveille, 1969) and of those lipids associated with oestrogen-induced lipidaemia (Ranney & Chaikoff, 1951). As a result of this and other metabolic changes the liver weight and liver lipid content increase when the non-laying pullet comes into lay. For example, Pearce (1971) found non-laying pullets to have a mean fresh liver weight of 23 g while 8 weeks later the laying hens had a mean liver weight of 40 g. The liver lipid concentration had increased from 6·3 to 14·2 %.

The results for both liver weight and oviduct weight are interesting since decrease in liver weight and the regression of the oviduct are both indicative of a cessation of the secretion of the gonadal hormones by the ovary. There is a marked possibility that the high dietary Cu level has suppressed oestrogen and possibly testosterone and progesterone production either at the level of the ovary or possibly by inhibition of follicle-stimulating hormone and luteinizing hormone production by the anterior pituitary. This would presumably result in a reversal of the liver weight and lipid content changes cited associated with the attainment of sexual maturity and egg-laying. The regression of the oviduct is similar to that observed when the hen ceases to lay due to natural moult or the onset of broodiness (Brown & Jackson, 1960). Unfortunately the state of the ovary was not observed. At slaughter the combs of the Treatment 5 and 6 hens were observed to be paler and less turgid than those of the other groups, indicating a decrease in androgen activity. The decreases in liver weight are of the same order as the increase in liver weight which occurs when the bird comes into lay (Pearce, 1971) and the decrease is presumably associated with a decrease in the liver lipid content, with the bird returning to a hormone status rather similar to that of the cockerel or the immature female fowl. As would be expected, a similar liver weight decrease was not found in female chicks given up to 720 mg Cu/kg diet as copper sulphate for 8 weeks (Norvell *et al.* 1974). The significant fall in liver DM content is to be expected in association with the loss of fat content resulting from decreased metabolic activity in the liver which would accompany the decrease in the demand of the hen for egg protein and lipid. However,

the possibility that the effects on the oviduct and liver are caused by decreased nutrient intake rather than by a direct effect on the endocrine system cannot be ruled out.

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