Effects of the protracted feeding of copper sulphate-supplemented diets to laying, domestic fowl on egg production and on specific tissues, with special reference to mineral content

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(*Received* 23 *January* 1979 – *Accepted* 2 *March* 1979)

1. Two experiments are reported. In both experiments a cereal-based diet containing 5 mg copper/kg was fed to two breeds of laying hens for 336 d. In Expt 1 four other groups were given this diet with the addition of $CuSO_4.5H_2O$ to give added levels of 200, 400, 600 and 800 mg Cu/kg diet. In Expt 2 the levels of added dietary Cu used were 100, 200, 300 and 400 mg/kg.

2. In Expt 1 records were kept of food intake, water intake, body-weight and egg production for eight 28 d periods and body-weight and egg number only were recorded for the full twelve periods. In Expt 2 full records, excluding water intake, were taken for all twelve periods.

3. Food and water intake showed a quadratic response to level of added dietary Cu, being enhanced at lower levels and depressed at higher levels of addition.

4. There was a quadratic response of total egg weight, mean egg weight and egg number to added dietary Cu. In Expt 1 egg number was maximum at 235 mg added Cu/kg diet for Warren Studler SSL (breed 1) and at 170 mg added Cu/kg diet for Shaver 288 (breed 2). In Expt 2 no breed effect occurred, the maximum egg number being calculated to occur at 176 mg added Cu/kg diet.

5. Depression of body-weight gain occurred at high levels of Cu addition. The depression of liver and oviduct weight found at high levels of addition appeared to be directly related to body-weight. A marked amount of feather loss also occurred at a high inclusion of $CuSO_4$ in the diet.

6. The reproductive systems of the hens did not appear to be adversely affected at the levels of additive used. Gross and microscopic examination of specific tissues revealed no pathological effects although gizzard and intestinal weights were increased and caecal weight decreased by high levels of added Cu. Those aspects of the blood chemistry examined did not reveal any consistent effect between the two experiments.

7. The liver Cu analyses indicate that between 600 and 800 mg added Cu/kg diet the liver Cu concentration rises sharply. Both liver Fe and Zn concentrations showed a positive linear response to added dietary Cu. In the kidney Cu and Zn concentrations were increased but only to a limited extent, while the concentration of Fe was unaffected.

The effects of adding copper compounds to the diet of the fowl have been briefly reviewed (Jackson, 1977). The growth and food conversion responses of adding Cu compounds to the diet of the growing bird have been statistically analysed (Fisher *et al.* 1971; Fisher, 1973). Their results indicated a quadratic response of body-weight gain and food conversion efficiency to added dietary Cu. This was clearly defined at higher levels due to growth depression, but the positive response at lower levels (up to 300 mg Cu/kg diet) was considered to be real. There was a maximum growth stimulation at approximately 170 mg Cu/kg diet and a maximum food conversion at 140 mg/kg.

In the broiler, Janssen (1971) and Fisher *et al.* (1973) found copper sulphate to cause lesions of the gizzard lining and Jensen & Maurice (1978) found the caeca of broilers given up to 250 mg additional Cu/kg diet to be distended and to contain dark, pasty contents with a Cu level of 5000 mg/kg dry matter (DM). They also found liver weight and the lipid

0007-1145/79/3278-0203 $01.00

content of liver DM to be increased. Bubien *et al.* (1971) found that giving a single dose of 800 mg Cu, as the sulphate, to adult male birds frequently caused death within 2 d, while 160 mg/d proved lethal after up to 34 d.

There have been few studies on the effect of added Cu as salts or oxides in the diet of the laying hen. In a recent study (Jackson, 1977) Cu, as sulphate, was added to the diet of laying hens up to a level of 1920 mg/kg diet.

Food and water intakes were depressed by 960 and 1920 mg/kg levels of added dietary Cu and water intake was increased by 240 mg added Cu/kg diet. Both food and water intakes showed a quadratic relationship with the level of added dietary Cu.

Body-weight gain was decreased 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 960 and 1920 mg added Cu/kg diet.

Liver and oviduct Cu and iron 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.

Egg production by hens given added Cu to a level of 960 mg/kg in the diet became infrequent and those given Cu at 1920 mg/kg ceased laying altogether after 14 d. However, the observations were only carried out for 35 d, which limits their usefulness.

Griminger (1977) found that Cu supplementation of the diet of laying hens with 2000 mg anhydrous $CuSO_4$ (796 mg Cu)/kg for 2 weeks depressed food intake, body-weight, egg production and eggshell thickness while 1000 mg Cu as sulphate/kg had no effect.

Jensen *et al.* (1978) found adding extra Fe, Cu, zinc, manganese and selenium to the diet to affect egg quality significantly, this being reflected in an increase in the Haugh units of the eggs.

Copper acetate, fed in a gelatin capsule to laying hens and providing 100 mg Cu daily caused weight loss, anaemia and death (Goldberg *et al.* 1956). Kuznetsov & Volkov (1974) fed $CuSO_4$ to laying hens for 270 d. They found 148 mg added Cu/kg diet to increase liver Cu by approximately 50% and plasma Cu by 36%, while liver and plasma Fe were also temporarily increased.

The studies reported in the present paper were designed to look at the longer-term effects of $CuSO_4.5H_2O$ fed as an additive in the diet of the laying hen, both in terms of productivity and of the effect of the additive on specific tissues of the fowl. Expt 1 investigated the effect of feeding $CuSO_4.5H_2O$ to a moderately high level (800 mg added Cu/kg) in the diet of the laying hen. Expt 2 was carried out to look in more detail at the effects of adding Cu, as sulphate, in the range 0–400 mg added Cu/kg diet.

EXPERIMENTAL

Expt 1

Seventy pullets, 17 weeks old and comprising equal numbers of Warren Studler SSL (breed 1) and Shaver 288 (breed 2) birds, previously vaccinated against Mareks disease, infectious bronchitis and epidemic tremors, were caged in galvanized-iron cages with individual feeders and water troughs. They were given a cereal-based control diet (Table 1) until the commencement of the experiment and subjected to a lighting programme of 17 h light–7 h darkness. The maximum temperature recorded in the poultry house during the experiment was 27° on 18 August 1976 and the minimum 14° on 11 January 1977. The Cu was added to the diets as finely ground $CuSO_4.5H_2O$. The fineness of the additive has previously been specified (Jackson, 1977). At 24 weeks old (2 August 1976), when all the birds were in lay, they were randomized into five treatment groups. The hens on treatment 1 were given the control diet and the hens in treatments 2–5 given this diet with the addition of 200, 400, 600 or 800 mg Cu/kg diet respectively. The appropriate experimental diet and

Table 1. *Expts* 1 *and* 2. *Composition* (*g/kg*) *and analysis of the control diet*

Constituent	
Ground maize	600·0
Ground wheat	74·2
Fish meal	26·1
Soya-bean meal	187·5
Grass meal	25·0
Limestone flour	71·9
Dicalcium phosphate	9·8
Common salt	3·0
Vitamin–mineral supplement (copper-free)*	2·5
Total	1000·0
Analysis	
Dry matter (g/kg)	894
Metabolizable energy (MJ/kg)	11·9†
Crude protein (nitrogen × 6·25) (g/kg)	162
Diethyl ether extract (g/kg)	26·1
Crude fibre (g/kg)	28·0
Calcium (g/kg)	33·6
Phosphorus (g/kg)	5·3
Copper (mg/kg)	5
Zinc (mg/kg)	106
Iron (mg/kg)	285

* The vitamin–mineral supplement provided (mg/kg diet): 2·4 retinol; 50 cholecalciferol; 3·4 riboflavin; 5 (μg) cyanocobalamin; 2·7 α-tocopherol; 1·5 menadione sodium bisulphite; 10 nicotinic acid; 4·5 Ca-D-panthothenate; 115 choline chloride; 40 Fe, 65 manganese; 0·6 iodine; 50 Zn; 0·1 selenium.

† Calculated.

water were available *ad lib.*, the latter containing 0·1 mg Cu/l. Food and water intakes were recorded daily for the first 2 weeks and thereafter food was recorded for each period and water on a weekly basis. Egg production was recorded daily and egg weight twice weekly. The full experimental results were recorded for eight 28 d periods. Body-weights and egg numbers only were recorded for a further four periods.

The birds were slaughtered by decapitation at the end of the twelfth period and blood analysed for packed cell volume (PCV), haemoglobin (Hb) and Cu contents, the latter by atomic absorption spectroscopy subsequent to wet ashing with concentrated nitric and perchloric acids (Thompson & Blanchflower, 1971). Blood serum was analysed for aspartate aminotransferase (AAT; *EC* 2.6.1.1) level by the method of Bergmeyer & Bernt (1965), one international unit referring to the oxidation of 1 μmol NADPH/min at 37°. The liver, kidneys, oviduct and gizzard were removed and weighed. The livers were analysed for lipid content by the method of Folch *et al.* (1957). The remainder of the digestive tract, including the caeca, was removed, washed internally with tap-water, lightly dried on blotting paper and the length and weight recorded. Liver and kidney samples were analysed for Cu, Fe and Zn content by atomic absorption spectroscopy after solution of the ash in dilute hydrochloric acid. The results were analysed for treatment and breed effects, the mean values for each breed being presented when interaction was found to occur.

Portions of the liver, kidney, spleen, adrenal glands, oviduct and ovary were fixed in buffered neutral formalin (100 ml/l), processed by standard methods and embedded in paraffin wax. The gizzard and a portion of gut from the anterior end of the small intestine were also treated in this way. Sections cut at 5 μm were stained by haematoxylin and eosin (H & E) and selected sections were stained by the rubeanic acid (dithio-oxamide) and by the 5-p-dimethylaminobenzylidine rhodamine method as outlined by Linquist (1969) for Cu and by the periodic acid-Schiff (PAS) method for glycogen.

Expt 2

One hundred and sixty-eight hens, 17 weeks old and comprising equal numbers of Warren Studler SSL (breed 1) and Shaver 288 (breed 2) were caged and fed as for Expt 1. At 24 weeks of age (2 August 1976) they were randomized and subjected to the following dietary treatments: control diet (treatment 1) as for Expt 1, and this diet to which was added 100, 200, 300 or 400 mg Cu/kg diet. Most of the hens were laying at the commencement of the experiment. Food intake, egg production, egg weight and body-weight were recorded as for Expt. 1 The recorded daily temperatures showed a maximum of 28° on 18 August 1976 and a minimum of 15° on 11 January 1977. After twelve 28 d periods the birds were slaughtered. Blood was analysed and organs removed and examined as for Expt 1, both total oviduct and shell gland being investigated. Assays were made of the Cu, Fe and Zn concentrations of the oviduct minus the shell gland.

RESULTS

Expt 1

One bird in treatment 2 died after 7 d. The death was not attributed to the experimental treatment and the missing-plot technique was applied. The two highest Cu treatments resulted in an obvious increase in feather loss by approximately 50% of the birds.

The results for water and food intake, food conversion, egg production and body-weight for Expt 1 are presented in Table 2. For the eight fully recorded periods body-weight gain showed a significant negative linear relationship ($P < 0.001$) with the dietary content of added Cu. The two groups being given the highest levels of added Cu showed almost no body-weight change, while the other three treatments caused an increase in body-weight which was greatest for treatments 1 and 2. The daily food intake records for the first 2 weeks of the experiment showed that treatment 2 birds had the highest food intake from the commencement of the experiment while the other three levels of Cu addition caused a food intake depression. Food intake was highest at 200 mg added Cu/kg (treatment 2) in all periods, a quadratic response in relation to added dietary Cu being evident in every period. There was a marked depression of intake at the two highest levels of added Cu. This food intake depression resulted in the Cu intake of the hens given diet 5 being increased only 7% above that of those offered diet 4, even though the level of Cu in diet 5 was one third greater than that in diet 4. Initially, the effect on water intake was a depression for the breed 2 birds receiving 800 mg added Cu/kg and an increase for the breed 1 birds receiving this diet. The total water intake showed a quadratic response ($P < 0.05$) to added dietary Cu, the average water intake for treatment 3 being highest in six periods and that for treatment 5 consistently the lowest in all periods.

In all eight periods there was a marked quadratic ($P < 0.001$ over all) response of both egg number and total egg weight to the level of added Cu. Egg number, but not total egg weight, showed a diet × breed interaction. The maximum egg number for the breed 1 and 2 hens was calculated to occur at 235 mg and 170 mg added Cu/kg diet respectively. Total egg weight was greatest for treatment 2 in seven of the eight periods, the maximum occurring at 191 mg added Cu/kg diet.

The response of mean period-mean egg weight to added dietary Cu was quadratic ($P < 0.05$). Treatment 2 mean egg weight was highest in seven of the eight periods while over-all treatment 5 mean egg weight was statistically lower than that of all other treatments. Statistical analysis showed the maximum mean period-mean egg weight to occur at 235 mg added Cu/kg diet.

Over-all food conversion efficiency (kg eggs/kg feed) showed a quadratic response ($P < 0.001$) to the level of added Cu. A diet × breed interaction was present. For both

Table 2. *Expt* 1. *Mean total food, water and supplementary copper intake, egg production, food conversion and body-weight of laying hens given Cu-supplemented diets**

No. of periods	Level of added Cu (mg/kg diet)	Diet no. 1: 0	2: 200	3: 400	4: 600	5: 800	Breed no. 1	Breed no. 2	SE of mean: Diet	Breed	Diet × Breed
8	Total supplementary Cu intake (g)	0	5·47	10·17	13·10	14·02	—	—	—	—	
	Food intake (kg)	26·10	27·35	25·42	21·83	17·53	25·69	21·61	0·685	0·433	
	Water intake (l)	59·19	66·13	69·39	62·77	53·75	70·53	53·97	4·190	2·650	
	Egg no.:										
	Breed 1	184·7	198·1	191·3	164·3	110·4	169·8	—	—	—	9·23
	Breed 2	186·4	192·5	177·6	132·1	54·7	—	148·7	—	—	
	Total egg wt (kg)	10·75	11·41	10·58	8·32	4·41	9·92	8·27	0·394	0·249	
	Mean period mean egg wt (g)	56·7	58·7	56·5	55·7	50·3	56·2	55·0	1·44	0·91	
	Efficiency of food conversion (kg eggs/kg food):										
	Breed 1	0·403	0·395	0·418	0·390	0·296	0·380	—	—	—	0·0179
	Breed 2	0·423	0·445	0·414	0·371	0·184	—	0·367	—	—	
	Initial body-wt (kg)	1·69	1·68	1·67	1·68	1·68	1·94	1·43	0·040	0·025	
	Body-wt change (g/28 d period)	41	37	27	−5	3	28	13	4·9	3·1	
12	Egg no.	261·6	280·1	266·4	220·8	133·1	244·7	220·1	10·20	6·45	
	Body-wt change (g/28 d period)	28	25	24	12	8	23	16	3·2	2·0	

* For details, see Table 1.

breeds there was no obvious evidence of any differences in food conversion between treatments 1, 2 and 3 (0, 200 and 400 mg/kg) but the mean value for treatment 4 (600 mg/kg) was generally poorer than for treatments 1, 2 and 3 while for treatment 5 (800 mg/kg) the efficiency of food conversion was significantly depressed ($P < 0.001$) in seven of the eight periods. The maximum food conversion efficiency was found to occur at 263 and 220 mg added Cu/kg diet for breeds 1 and 2 respectively.

Over the twelve periods egg number and body-weight gain showed the same general trends as over the first eight periods. Egg number was greatest for treatment 2 for ten of the twelve periods. Treatment 2 gave a response of 7% over the control and the hens in treatments 4 and 5 showed a depression in egg number of 16 and 50% respectively compared to the control.

The mean blood Hb, PCV, Cu and serum AAT values at slaughter are presented in Table 3. The mean blood Hb for treatment 5 was significantly less than for treatments 2 and 3. PCV showed a quadratic relationship with level of added Cu, the values for treatments 2 and 3 being significantly ($P < 0.001$) greater than for treatment 1. The blood Cu analyses of variance showed no significant differences. The mean serum AAT value was highest for treatment 3 but no statistically significant difference was present.

Liver and kidney fresh and dry weights and DM results are given in Table 4 together with the liver lipid concentrations and total contents, while the Cu, Fe and Zn values for these organs are presented in Table 5.

Liver fresh and dry weights and kidney dry weight showed a negative linear response ($P < 0.001$, $P < 0.001$ and $P < 0.05$ respectively) to the level of added Cu, the mean values being increased, although not significantly, by the lowest (200 mg/kg) level of added Cu. The higher levels of added Cu caused a statistically significant depression of the dry weights of the liver and kidney, but the mean kidney fresh weight was higher for all Cu treatments than for the control, although the effect was not statistically significant.

Liver fresh weight, as g/kg body-weight, was highest for treatment 2, which was significantly higher than for treatment 3 ($P < 0.001$) and treatment 5 ($P < 0.01$). Kidney fresh weight (g/kg body-weight) had a linear relationship to added dietary Cu ($P < 0.01$) and was increased above the control value by all Cu treatments. Liver DM content had a negative linear relationship ($P < 0.001$) to the level of added Cu, being highest for treatment 2 and significantly depressed below the control value in treatments 3 and 5. Kidney DM (mg/g) had a negative linear relationship ($P < 0.05$) with the level of added Cu, all the mean values being lower than for the control, the effect being statistically significant for treatments 4 and 5.

The liver lipid concentration and total content decreased linearly (both $P < 0.001$) with the level of added dietary Cu.

Entire and lipid-free liver DM Cu concentrations (Table 5) were increased by added dietary Cu ($P < 0.001$), showing a quadratic relationship ($P < 0.001$), treatment 5 being significantly higher ($P < 0.001$) than all other treatments. This concentration effect was reflected in total liver Cu content, the quadratic effect being highly significant.

The effect of dietary added Cu on liver DM Fe concentration was linear ($P < 0.05$), but the total Fe content of the liver DM was not affected by the diet.

The Zn concentration in liver showed a positive linear relationship to added dietary Cu ($P < 0.001$), although only treatment 5 Zn content was significantly greater than for treatment 1. The total liver Zn content was not affected.

Kidney Cu concentration was increased linearly ($P < 0.001$) by added dietary Cu although total content was not affected. Kidney Fe concentration and content were not affected while Zn concentration increased linearly ($P < 0.001$) and total Zn content was not affected by added dietary Cu.

The oviduct, gizzard, intestinal and caecal results appear in Table 6. There was a

Table 3. *Expt* 1. *Mean blood haemoglobin (Hb), packed cell volume (PCV), copper and serum aspartate aminotransferase (AAT;* EC 2.6.1.1) *at slaughter for laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
	1	2	3	4	5				
Level of added Cu (mg/kg) ...	0	200	400	600	800	1	2	Diet	Breed
Hb (g/l)	82·8	89·6	88·9	83·5	78·6	84·3	85·1	3·03	1·91
PCV	0·23	0·29	0·29	0·25	0·25	0·27	0·26	0·010	0·006
Cu (μg/l)	304	283	275	316	286	302	283	20·7	13·1
Serum AAT (iu†/l)	224	224	276	247	226	251	227	22·5	14·3

* For details, see Table 1.
† One international unit refers to the oxidation of 1 μmol NADPH/min at 37°.

Table 4. *Expt* 1. *Liver and kidney fresh and dry weight, dry matter* (DM) *concentration and liver lipid levels for laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
	1	2	3	4	5				
Level of added Cu (mg/kg) ...	0	200	400	600	800	1	2	Diet	Breed
Liver wt (g):									
Fresh	43·9	48·4	34·1	38·2	31·6	43·2	35·3	2·37	1·50
Dry	16·6	19·8	11·0	13·1	9·7	15·4	12·6	1·29	0·82
Fresh (g/kg body-wt)	22·0	24·7	18·4	22·5	19·8	20·1	23·0	1·25	0·79
Liver DM (mg/g)	369	402	317	334	306	343	348	13·3	8·4
Liver lipid content (mg/g DM)	364	427	254	266	202	304	300	27·3	17·2
Total liver lipid content (g)	6·5	9·0	3·1	3·9	2·1	5·5	4·3	0·88	0·56
Kidney wt (g):									
Fresh	10·4	12·0	11·3	10·9	11·3	11·3	11·0	0·69	0·43
Dry	3·0	3·4	3·0	2·8	2·7	3·1	2·9	0·19	0·12
Fresh (g/kg body-wt)	5·30	6·24	6·05	6·57	7·22	5·31	7·24	0·398	0·252
Kidney DM (mg/g)	294	282	266	254	242	269	266	12·4	7·9

* For details, see Table 1.

negative linear relationship ($P < 0.05$) between oviduct fresh weight and level of added Cu, but this effect is lost when the results are expressed per kg body-weight.

Gizzard fresh weight, both the absolute (g) and relative (g/kg body-weight) values, showed a positive linear relationship with added dietary Cu ($P < 0.05$ and $P < 0.001$ respectively).

Intestinal fresh weight showed a marked linear ($P < 0.01$) increase in response to added Cu. This effect was not seen for absolute length but was present for length per unit body-weight. Caecal weight and length per kg body-weight showed a marked ($P < 0.001$) linear relationship to the added dietary Cu.

Examination of the koilin layer of the gizzard showed no abnormalities either grossly or histologically in any of the birds and the underlying epithelium was intact. Inflammatory or degenerative changes were not seen in the gizzards and examination of the various tissues from birds fed the $CuSO_4$-containing diets showed no significant changes from the tissues of the control birds. Fatty changes were found in the liver of many of the birds and identified by vacuoles in the hepatic cell cytoplasm, and varying small amounts of glycogen were also observed in the hepatic cells. However, these changes showed no correlation with diet.

Table 5. *Expt* 1. *The copper, iron and zinc concentration (μg/g) and contents (μg) in liver and kidney of laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
Level of added Cu (mg/kg diet) ...	1 0	2 200	3 400	4 600	5 800	1	2	Diet	Breed
Liver dry matter									
Concentration:									
Cu	9·5	10·5	29·6	40·3	267·0	48·4	94·4	31·24	19·76
Fe	262	234	376	377	395	336	321	50·2	31·7
Zn	103	88	158	154	228	164	129	27·8	17·6
Total content:									
Cu	150	196	299	444	2445	480	934	364·1	230·3
Fe	4042	4638	3700	4494	3743	4536	3710	517·7	327·4
Zn	1610	1728	1509	1875	2211	2068	1505	268·6	169·9
Liver lipid-free dry matter									
Concentration:									
Cu	14·9	18·8	39·2	53·0	336·6	61·9	123·1	39·67	25·09
Fe	406	422	486	511	483	475	449	58·4	37·0
Zn	161	160	205	215	281	227	181	33·9	21·5
Kidney dry matter									
Concentration:									
Cu	13·6	13·4	15·6	16·2	17·9	14·9	15·8	0·79	0·50
Fe	238	242	252	255	314	246	273	29·6	18·7
Zn	106	114	122	131	130	122	119	5·7	3·6
Total content:									
Cu	40·5	44·8	45·7	44·9	46·7	44·7	44·3	2·50	1·58
Fe	719	809	748	704	902	756	797	111·6	70·6
Zn	319	381	386	365	349	379	341	36·2	22·9

* For details, see Table 1.

Table 6. *Expt* 1. *Oviduct and gizzard weights, intestinal and caecal lengths and weights of laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
	1	2	3	4	5				
Added dietary Cu (mg/kg) ...	0	200	400	600	800	1	2	Diet	Breed
Fresh wt:									
Oviduct (g)	60·8	59·9	55·4	57·7	49·7	59·5	53·9	3·59	2·27
(g/kg BW)	31·3	31·3	30·3	34·5	31·5	28·1	35·4	2·17	1·37
Gizzard (g)	21·8	22·3	22·0	24·9	23·4	25·3	20·4	0·84	0·53
(g/kg BW)	10·9	11·5	11·9	14·8	14·5	12·0	13·4	0·45	0·28
Intestine (g)	27·8	25·3	29·7	30·4	32·8	34·1	24·3	1·48	0·94
(g/kg BW)	13·9	13·3	15·3	19·1	21·3	16·2	17·0	1·04	0·66
Caecum (g)	5·2	4·5	4·7	5·4	4·9	5·8	4·1	0·19	0·12
(g/kg BW)	2·6	2·3	2·5	3·4	3·2	2·7	2·8	0·12	0·07
Length:									
Intestine (mm)	492	486	466	497	483	507	463	20·6	13·0
(m/kg BW)	0·250	0·253	0·256	0·299	0·310	0·240	0·307	0·0152	0·0096
Caecum (mm)	58	55	55	61	61	62	54	2·6	1·7
(mm/kg BW)	29	29	30	37	39	29	36	1·8	1·2

BW, body-wt.

* For details, see Table 1.

Using the staining techniques the presence of Cu was demonstrated only in the livers of birds given the diet containing 800 mg added Cu/kg diet.

Expt 2

None of the birds died during the course of the laying trial.

The body-weight and egg production results are shown in Table 7. Body-weight gain showed a significant quadratic relationship ($P < 0.01$) with the dietary content of added Cu. All treatments resulted in a body-weight increase. This was greatest for 200 mg added Cu/kg diet and for treatment 5 was significantly depressed below all the other mean values. There was a quadratic relationship ($P < 0.05$) between food intake and the level of added Cu in the diet, the intake for treatment 5 being significantly depressed ($P < 0.05$) below that for treatments 3 and 4. The mean food intake by treatment 3 or 4 hens was higher than for the other treatments in ten of the twelve periods.

There was a quadratic relationship between egg number and added dietary Cu for periods 3, 4 and 5. The number of eggs produced by the birds on the highest level of dietary Cu addition (400 mg Cu/kg diet) was consistently the lowest. For the full experimental period the value for treatment 3 (200 mg/kg) was significantly higher ($P < 0.05$) than for treatment 5 (400 mg/kg) and statistical analysis showed the maximum egg number to occur at 176 mg added Cu/kg diet.

The over-all analysis showed that treatment 3 (200 mg/kg) gave the highest total egg weight (maximum for eight of the periods) and treatment 5 (400 mg/kg) the lowest (minimum for eleven of the periods), the values for treatments 3 and 4 being statistically greater ($P < 0.05$) than for treatment 5 (400 mg/kg). Calculation of the total egg weight response to added Cu gave a maximum at 151 mg added Cu/kg diet. Mean egg weight was highest for treatment 3 and lowest for treatment 5 and the maximum was found at 141 mg added Cu/kg diet.

Treatments 3 and 4 had the best mean efficiency of food conversion (maximum at 161 mg added Cu/kg diet) while that for treatment 5 was the poorest, but none of the differences attained statistical significance.

The mean blood Hb, PCV, Cu and serum AAT results are presented in Table 8. The Hb concentration and PCV were higher for all the Cu-treated groups than for the control, this effect attaining significance for treatment 4 Hb (300 mg/kg). There was a linear relationship ($P < 0.001$) between blood Cu and level of added dietary Cu, the values for treatments 4 and 5 being significantly greater ($P < 0.001$ and $P < 0.05$ respectively) than for the control.

Serum AAT showed a negative linear ($P < 0.01$) relationship to level of added dietary Cu, the value at the highest level of added Cu being significantly lower ($P < 0.05$) than for treatments 1 and 2.

Liver and kidney fresh and dry weights and DM results are given in Table 9 together with the oviduct and shell gland weights and the liver lipid values. Fresh and dry liver weights and liver DM content showed a negative linear ($P < 0.001$) response to level of added Cu, and liver fresh weight per kg body-weight showed a quadratic response ($P < 0.05$), treatment 1 being significantly greater than all the other mean values. No such response was observed for kidney except as fresh kidney weight per kg body-weight when a linear response was noted ($P < 0.05$), the value for treatment 3 being lowest. As in Expt 1 the liver lipid concentration and total content decreased linearly (both $P < 0.001$) with the level of added dietary Cu.

The Cu, Fe and Zn results are given in Table 10. Only the concentration values are presented. As in Expt 1 liver DM Cu concentration showed a quadratic ($P < 0.001$) relationship to the level of added dietary Cu. The Fe and Zn concentrations of the liver

Table 7. *Expt 2. Mean total food and supplementary copper intake, egg production, food conversion and body-weight of laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
Level of added Cu (mg/kg diet) ...	1 0	2 100	3 200	4 300	5 400	1	2	Diet	Breed
Total supplementary Cu intake (g)	0	3·49	7·16	10·59	13·41	—	—	—	—
Food intake (kg)	35·06	34·94	35·83	35·31	33·51	36·49	33·38	0·629	0·398
Egg no.	263·3	259·8	272·4	270·4	249·5	264·4	261·8	8·01	5·07
Egg wt (kg)	15·38	15·13	15·73	15·54	14·01	15·40	14·91	0·492	0·311
Mean egg wt (g)	57·0	56·4	57·7	56·4	56·3	58·2	55·3	1·103	0·698
Efficiency of food conversion (kg eggs/kg food)	0·434	0·429	0·439	0·439	0·417	0·421	0·442	0·0113	0·0072
Initial body-wt (kg)	1·68	1·66	1·65	1·64	1·67	1·88	1·44	0·028	0·018
Body-wt change (g/28 d period)	26	31	28	23	15	29	19	2·8	1·7

* For details, see Table 1.

Table 8. *Expt 2. Mean blood haemoglobin (Hb), packed cell volume (PCV), copper and serum aspartate aminotransferase (AAT*; EC 2.6.1.1) *for laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
Level of added Cu (mg/kg) ...	1 0	2 100	3 200	4 300	5 400	1	2	Diet	Breed
Hb (g/l)	80·3	87·6	83·1	93·2	88·7	84·4	88·8	3·84	2·43
PCV	0·26	0·27	0·26	0·29	0·28	0·27	0·28	0·011	0·007
Cu (μg/l)	266	279	296	324	307	292	297	11·3	7·2
Serum AAT (iu†/l)	230	224	208	197	187	204	214	12·0	7·6

* For details, see Table 1.

† One international unit refers to the oxidation of 1 μmol NADPH/min at 37°.

DM increased linearly ($P < 0·001$ and $P < 0·01$ respectively) with increasing added dietary Cu.

In the kidney DM Cu concentration showed a dietary effect ($P < 0·001$), the relationship being cubic. The kidney Fe concentration was not affected and kidney Zn concentration increased linearly ($P < 0·001$) with added dietary Cu.

Treatment 5 oviduct weight (Table 9) showed a significant depression below the mean values of treatments 1 and 4. On a body-weight basis treatment 4 had the heaviest oviduct weight, this being significantly greater than for treatment 2 ($P < 0·001$) and treatment 3 ($P < 0·05$). Oviduct DM content was not affected by treatment.

Oviduct DM, Cu and Fe concentrations both showed a quadratic relationship ($P < 0·01$) to dietary added Cu, while oviduct Zn concentration was not affected. Shell gland weight (Table 9) was not affected by diet. Gross and histological examination of the specific tissues investigated revealed no changes which could be attributed to dietary treatment.

Table 9. *Expt 2. Liver, kidney and oviduct fresh and dry weight, dry matter* (DM) *concentration and liver lipid levels for laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
Level of added Cu (mg/kg diet) ...	1 0	2 100	3 200	4 300	5 400	1	2	Diet	Breed
Liver wt (g):									
Fresh	42·6	39·0	36·3	37·3	33·6	39·8	35·7	1·57	0·99
Dry	15·8	14·0	12·5	12·7	10·7	13·7	12·6	0·87	0·55
Fresh (g/kg BW)	21·8	19·6	18·4	19·9	18·8	17·8	21·6	0·67	0·42
Liver DM (mg/g)	366	350	335	331	311	334	343	8·63	5·46
Liver lipid content (mg/g DM)	383	344	288	292	249	301	321	17·7	11·2
Total liver lipid content (g)	6·5	5·3	4·0	4·1	3·0	4·6	4·5	0·57	0·36
Kidney wt (g):									
Fresh	10·4	10·5	10·1	11·3	10·3	10·2	10·8	0·31	0·20
Dry	2·69	2·65	2·58	2·86	2·54	2·65	2·68	0·082	0·052
Fresh (g/kg BW)	5·40	5·58	5·22	6·14	5·97	4·61	6·72	0·262	0·166
Kidney DM (mg/g)	257	254	256	253	248	259	249	4·35	2·75
Oviduct wt									
(g)	57·0	53·5	55·2	58·5	52·7	55·2	55·5	1·79	1·13
(g/kg BW)	29·6	26·7	28·5	31·9	30·5	25·0	33·9	1·06	0·67
Shell gland wt									
(g)	16·2	15·9	15·3	16·0	15·9	15·6	16·0	0·62	0·39
(g/kg BW)	8·5	7·9	7·8	8·7	9·2	7·0	9·8	0·36	0·23

BW, body-wt.
* For details, see Table 1.

Table 10. *Expt 2. The copper, iron and zinc concentration (μg/g) in liver, kidney and oviduct* DM *of laying hens given Cu-supplemented diets**

	Diet no.					Breed no.		SE of mean	
Level of added Cu (mg/kg diet) ...	1 0	2 100	3 200	4 300	5 400	1	2	Diet	Breed
Liver dry matter Concentration:									
Cu	10·2	12·0	13·8	14·5	26·3	14·6	16·1	1·18	0·74
Fe	250	291	323	297	407	336	292	26·5	16·8
Zn	102	102	118	109	141	118	111	8·2	5·2
Liver lipid-free dry matter Concentration:									
Cu	16·4	17·7	19·1	20·4	34·3	20·4	22·8	1·27	0·80
Fe	400	438	452	421	533	476	422	33·3	21·1
Zn	165	153	163	152	185	166	161	9·2	5·8
Kidney dry matter Concentration:									
Cu	14·7	21·4	14·7	17·6	20·2	17·4	18·1	0·98	0·62
Fe	254	262	250	274	288	251	280	13·0	8·2
Zn	105	106	108	111	118	108	111	2·4	1·5
Oviduct dry matter Concentration:									
Cu	6·3	12·0	9·3	10·6	8·1	8·7	9·8	1·03	0·65
Fe	41	56	56	65	50	51	56	6·6	4·2
Zn	47	47	47	48	47	45	49	1·9	1·2

* For details, see Table 1.

DISCUSSION

The tolerance of the laying hen to the high levels of dietary Cu used in Expt 1 over the fairly long-term period involved and to the even higher levels used previously (Jackson, 1977) was unexpected and in contrast to the low tolerance shown by the ruminant (Shand & Lewis, 1957; Todd & Thompson, 1963) and even by the pig (Suttle & Mills, 1966*a*, *b*).

The increase in food intake at the lower levels of Cu intake observed in the initial experiment of this series (Jackson, 1977) is again evident, as is the depression of food intake at the higher levels of addition, a 33% fall in food intake being observed at the highest level. Since the diets were isonitrogenous and isocaloric, the marked depression of food intake, especially at the highest level of Cu addition, is apparently due to the presence of the Cu. The current work does not help clarify whether this effect is due to Cu toxicity or to palatability. Even though the domestic fowl is reported to have relatively few taste buds compared with other species (Kare & Rogers, 1976) it cannot be ruled out that the quadratic effect of Cu on food intake may be due, at least partially, to a palatability effect. The results for body-weight gain showed that the higher levels of added Cu (400–800 mg/kg) caused a definite decrease while the lower levels had no marked effect. The linear decrease of body-weight gain found in Expt 1 agrees with the results of Jackson (1977) and Goldberg *et al.* (1956) for hens. In the former work a considerable loss of body-weight was attributed to liver weight loss and oviduct regression, but in the current experiments these were not important factors. In Expt 1 the decrease of body-weight gain at 600 and 800 mg added Cu/kg is clearly due to the decrease of food intake (16 and 33% respectively). From the first day of Expt 1 the 200 mg added Cu/kg level had a small stimulatory effect and the higher levels a definite negative effect on intake. Although for the first 2 weeks the presence of added $CuSO_4$ in the diet had opposite effects on water intake for the two breeds of hens used, overall the highest level used caused a depression of water intake. This effect agrees with that observed previously (Jackson, 1977).

The quadratic response of egg number and total egg weight to dietary $CuSO_4$ in Expt 1 was apparent to a lesser extent in Expt 2, although there is no obvious explanation as to why the breed effect for egg number found in the first experiment was not present in the second experiment. The results substantiate the production values presented by Jackson (1977) based on a small number of hens given Cu-containing diets for a short (5 weeks) period.

Examination of the ovaries, oviducts and the laying records of the hens in both experiments showed that treatment had no effect on the number of birds found to have an inactive reproductive system. However, in Expt 1, once the hens were introduced to the Cu-containing diets those on treatment 2 showed an immediate and sustained increase in egg number and those on treatments 4 and 5 showed a very definite decrease. The effect in these two groups was fairly consistent for each bird and not due to complete cessation of laying by a proportion of the birds. The effect found at 600 and 800 mg added Cu/kg diet is similar to that found previously (Jackson, 1977) when at 960 mg/kg production became infrequent, but differs from the effect at 1920 mg/kg when laying ceased after a short time.

In both experiments the 200 mg/kg level gave the highest mean egg weight, while at the highest level used a depression was evident. However, it is not clear why the maximum in Expt 1 occurred at 235 mg added Cu/kg diet while in Expt 2 the maximum point of the response curve was at 141 mg added Cu/kg diet.

As for egg number, the breed effect for efficiency of food conversion for egg production found in Expt 1 (maximum for breed 1, 263 mg added Cu/kg diet; for breed 2, 220 mg added Cu/kg diet) was not present in Expt 2 (maximum 161 mg added Cu/kg diet). The reason for the maximum response occurring at a lower level of $CuSO_4$ addition in

the latter experiment is not readily explained but could be due to various environmental differences between the experiments. That the positive effect of added dietary $CuSO_4$ on egg production may be similar to that observed when antibiotics are used as food additives is not supported by the intestinal weight and length values, which increase when presented on a unit body-weight basis, while antibiotics in the diet have been shown to decrease intestinal weight and thickness in growing fowl (Gordon, 1952; March & Biely, 1967) and pigs (Braude *et al.* 1955; Taylor & Harrington, 1955). King (1972) gave diets containing added Cu (100 mg as sulphate/kg) to chicks and found decreased small intestinal weight which was accompanied by increased caecal weight, this latter effect being observed in Expt 1. Nevertheless the response of the intestine to high dietary $CuSO_4$ in the mature hen need not necessarily be the same as the response found to lower levels in the younger growing animal.

The results for blood Cu content were not consistent in that in Expt 1, as in a previous experiment (Jackson, 1977), no significant differences occurred, while in Expt 2 the 300 and 400 mg/kg levels caused a small but statistically significant increase over the control value. Goldberg *et al.* (1956) and Kuznetsov & Volkov (1974) found that Cu salts in the diet of adult hens caused increased blood Cu concentration. The conditions in the latter work were not dissimilar to the present experiments in that it involved long-term feeding (270 d) of the $CuSO_4$-containing diet. The results tend to support the theory that, as for Fe, the animal has a barrier mechanism for limiting its Cu absorption.

The depressed Hb for treatment 5 compared to the values for treatments 2 and 3 in Expt 1 indicates some agreement with the results of Ruszczyc *et al.* (1962) and Kirchgessner *et al.* (1970) for the chick, although the very different hormonal status of the chick and laying hen and the long-term feeding period in the present experiments rather invalidate any comparison.

In Expt 1 no definite effect on serum AAT could be identified although in Expt 2 the evidence strongly indicated a depressing effect of dietary Cu on serum AAT level, but there was no evidence of the elevated serum AAT levels associated with stress or dysfunction of liver in the human (Norberg, 1961) or sheep (Todd & Thompson, 1963).

The depression of liver weight in response to dietary Cu appears to be related to the lower body-weight, although when expressed on a unit body-weight basis there was still some indication of a depression, especially in Expt 2. The liver lipid content was depressed in both experiments as a result of high dietary Cu, indicating a possible depression of fatty acid and lipid synthesis, which in the fowl occur mainly in the liver (Ranney & Chaikoff, 1951; Goodridge, 1968; O'Hea & Leveille, 1969). The fact that, on a body-weight basis, oviduct weight was not depressed by Cu level does not suggest that there was any major effect on the secretion or action of the gonadotrophins or the steroid hormones associated with normal development of the reproductive system. Any effect on egg production is probably associated with increased or decreased nutrient intake and efficiency of food conversion rather than with an effect on the reproductive endocrine system.

The results of the staining technique for Cu (Lindquist, 1969) agree with the quantitative results. The liver Cu concentrations reported here and previously (Jackson, 1977) suggest that there is a critical point between 600 and 800 mg added Cu/kg at which liver Cu concentration rises sharply.

The liver Fe and Zn concentrations in both experiments showed a positive linear response to increasing dietary Cu. The increased liver Fe is contrary to the results found in the pig, where high levels of dietary Cu caused reduced Fe storage (Cassidy & Eva, 1958; Ritchie *et al.* 1963) and is at variance with the anaemia observed in chicks given a high level of dietary Cu (Ruszczyc *et al.* 1962), but does agree with the increase found previously for the mature bird (Jackson, 1977). The increase in liver Zn concentration is in conformity with

the observation that Cu has a Zn-sparing effect in the pig (Wallace *et al.* 1960; Ritchie *et al.* 1963) although a contrary result has been found by others (O'Hara *et al.* 1960; Suttle & Mills, 1966*b*).

The results both of the laying trial and the associated observations and those obtained by gross and histological examination of the various tissues indicate that at the lower levels used the presence of $CuSO_4$ in the diet for an extended period of time has a beneficial effect on production and food conversion, and although these factors were adversely affected at high levels of addition there was no definite evidence of pathological changes, the increased gizzard, intestine and caecal weights indicating a response to the presence of the $CuSO_4$.

The loss of feathers noted in Expt 1 was more prevalent in the white (Shaver 288) than in the brown (Warren Studler SSL) birds and could markedly increase heat loss (O'Neill & Jackson, 1974).

The authors thank Mrs R. Park and Mr P. Shearer for technical assistance, Dr S. T. C. Weatherup of the Biometrics Division for assistance with statistical analyses and Dr R. M. McCracken for the histological examinations.

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Printed in Great Britain